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Award Number: DAMD17-98-1-8155

TITLE: Synthesis of Epothilone Analogs: Toward the Development of Potent Anticancer Drugs

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New York, New York 10021

REPORT DATE: August 2002

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 2002	Annual Summary (1 Jan 99 - 31 Jul 02)

4. TITLE AND SUBTITLE
Synthesis of Epothilone Analogs: Toward the Development of Potent Anticancer Drugs

5. FUNDING NUMBERS
DAMD17-98-1-8155

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
report contains color

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*
Epothilones are cytotoxic natural products that inhibit the growth of cancer cells by the same tubulin-stabilizing mechanism of Taxol. We have been developing a program in epothilone-based chemotherapy, and have succeeded in accomplishing efficient synthetic protocols to access large amounts of important analogs quickly. Our lead candidate, 12,13-desoxyepothilone B (dEpoB), is currently in Phase I clinical trials. To further develop and screen a variety of strong back-up candidates in our clinical development program, we have made and tested a series of dehydro-desoxy-epothilones. In the process, we have also discovered an even more practical synthetic process toward the synthesis of our lead drug candidate, dEpoB. We have also examined the scope of synthetic modifications to the natural epothilone structure with regards to biological activity. These experiments include the expansion of the ring-size of the epothilones and introduction of trifluoromethyl groups. These studies demonstrate that the dehydroepothilones are viable cytotoxic agents, and that minimal ring-expansion strategies maintain the biological activity of the epothilones.

14. SUBJECT TERMS
breast cancer, epothilone, desoxyepothilone B, anticancer agent, analog synthesis, back-up candidates, cytotoxicity, tubulin binding

15. NUMBER OF PAGES
67

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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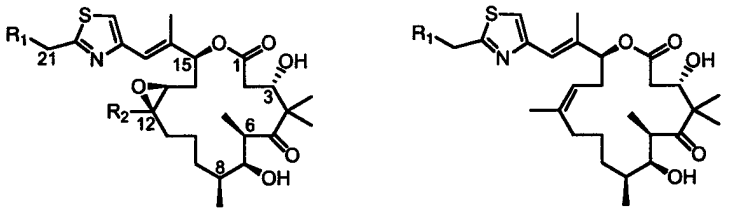
INTRODUCTION

Microtubules are dynamic, polymeric structures that are an integral part of all eukaryotic cells.[1] During mitosis, the dynamics of microtubule polymerization and depolymerization are finely controlled and any variation in the rate of polymerization can profoundly affect cellular replication. Affected cells are unable to pass through a checkpoint in the cell cycle and enter into programmed cell death. There are two major classes of chemotherapeutic agents that induce mitotic arrest by disrupting microtubule dynamics; those that depolymerize tubulin and those that stabilize tubulin polymers. The most well known of tubulin-stabilizing agents is Taxol® (paclitaxel), which is currently a front-line anticancer agent.[2] However, limitations due to multidrug-resistance (MDR) susceptibility and lack of aqueous solubility render it less than an ideal drug. The latter condition compels recourse to formulation vehicles such as cremophores, which have their own associated toxicities. Given the well-established usefulness of Taxol® against a variety of oncological indications, it is not surprising that there has been a continuing interest in agents which share the mechanism of action of Taxol® while offering potential therapeutic advantages. One target of opportunity would be the discovery of a drug, operating in the mechanistic framework of Taxol®, which exhibits a much greater robustness toward disablement via the onset of multidrug resistance.

In 1993, the cytotoxic macrolide natural products, epothilone A (EpoA) and B (EpoB), along with other minor related constituents were isolated from the myxobacterium *Sorangium cellulosum* (Figure 1).[3] In 1995, scientists at Merck discovered that the epothilones kill cells through a "Taxol®-like" mechanism of action.[4] However, in contrast to Taxol®, the epothilones inhibited the growth of MDR cell lines raising the hope that epothilone-derived anticancer drugs might eventually be useful for the treatment of drug-resistant tumors.[5] Our laboratory accomplished the first total syntheses of epothilone A and B,[6] and was soon joined by others. [7] Our multidisciplinary research program resulted in the discovery that the 12,13-desoxyepothilones (Figure 1, **1** and **2**) are less toxic than their "epoxy" analogues, which are currently in clinical studies. On the basis of its extensive and highly encouraging package of pre-clinical data in mice and dogs, dEpoB (**1**, Figure 1) has been advanced to Phase I clinical trials at UCLA and at Memorial Sloan-Kettering Cancer Center.[8,9]



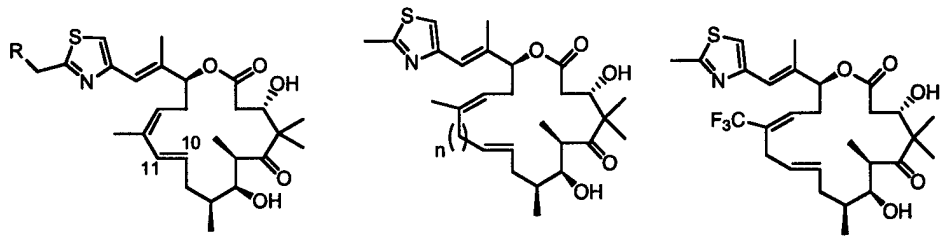
R_1, R_2 = H, epothilone A
R_1 = H, R_2 = CH_3, epothilone B
R_1 = OH, R_2 = CH_3, epothilone F

1 R_1 = H, 12,13-desoxyepothilone B
2 R_1 = OH, 12,13-desoxyepothilone F

Figure 1. Structures of the epothilones.

The research described here involves the design and synthesis of a number of new analogues of dEpoB for the purpose of generating *potent back-up candidates* in our anticancer chemotherapy program. Mindful of potential benefits of exploring new organic chemical methodologies, we re-examined our total synthesis endeavors in the epothilone arena and sought to devise novel routes to assembling these structures. These investigations led to a more efficient synthesis of our lead candidate dEpoB, as well as the generation of new analogues, which have

been shown to be highly cytotoxic in cell-culture assays. These analogues encompass the 10,11-dehydro versions of dEpoB and dEpoF (**3** and **4**, Figure 2) and a ring-expanded version (**6**) of the 26-trifluoro compound which was originally described in the Statement of Work. As will be discussed later, the 26-trifluoro analogue of dEpoB could not be accessed by our current technologies. Ring-expanded epothilone congeners (**5a**, **5b**), however, could easily be synthesized by our new route, and are also described. These studies lay down the guidance for further structure-activity level explorations of the epothilones, for the creation of newer, more effective anticancer therapies.



3, R = H, 10,11-dehydro-dEpoB (epothilone 490)
4, R = OH, 10,11-dehydro-dEpoF

5a, n = 1, [17]ddEpoB
5b, n = 2, [18]ddEpoB

6, 27-CF_3-[17]ddepoB

Figure 2. Structures of new epothilones synthesized and evaluated in this work.

Of the remaining compounds proposed in the original Statement of Work, the synthesis of the 21-amino analogue of dEpoB was not undertaken. This was largely because other workers had reported progress in that arena, as is often the case in a competitive research project. It was felt that the research scope would be better served by undertaken the synthesis and evaluation of newer, unexamined epothilones, to better augment our drug discovery program in epothilone-based anticancer chemotherapy.

BODY: **Chemical Synthesis and Anticancer Evaluation of New Epothilones**

1. Synthesis of 10,11-dehydro-dEpoB (Epo490). Epo490, which contains a 10,11 *E*-olefin conjugated to the usual 12,13-unsaturation of dEpoB, showed highly favorable *in vitro* cytotoxicity in preliminary screens.[10] The prospects for a quality synthesis of epo490 from dEpoB were not inviting. From a purely synthetic perspective, the presence of the C10-C13 diene unit was suggestive of potential forays into new ways of macrocyclization toward the synthesis of epothilones. Olefin metathesis certainly stands out as a premier methodological advance in carbon-carbon bond formation reactions of recent vintage.[11] Unfortunately, ring-closing olefin methathesis directed to the 12,13 linkage has invariably led to a stereorandom mixture of olefin isomers, separable only with the greatest of difficulty.

From this background, we considered the possibility of using RCM to construct a 10,11-double bond *in a substrate which already contains the putative 12,13-unsaturation.* Successful olefin metathesis would produce a 10,11;12,13-diene of the type found in epo490. We also hoped that position-selective hydrogenation of the disubstituted 10,11-olefin would provide access to the 12,13-desoxyepothilones themselves. As seen below, this new strategy for synthesizing epothilones has been realized. When combined with a new and highly convergent way to join key pre-epothilone fragments, a highly modular synthesis from readily available units has been achieved.

The synthetic plan envisaged a construction of a "seco" acyclic triene (**9**, Figure 3) positioned for diene-ene RCM for macrolide formation. Fortunately, we could draw upon previously disclosed and highly accessible building blocks to pursue a new vision of the epothilone synthesis problem. These are vinyl iodide **7**[12] and aldehyde **8**.[13] "Seco" compound **9** could be accessed from a reassembly of advanced synthetic intermediates (Figure 3). The C11 - C15 domain can be acylated with an appropriate C1 acid moiety to construct the C1-C15 ester linkage. The stereoselective formation of the C3 stereocenter developed into a major challenge in earlier efforts. Investigations revealed that best yields were obtained from a chiral titanium-mediated aldol reaction with aldehyde **8**, affording the correct C3 alcohol, *after* construction of the C6, C7 and C8 stereocenters.[13] For the synthesis of our cyclization precursor, acylation with acetic anhydride to generate the C15 acetate (*vide infra*), followed by a diastereoselective aldol reaction with **8** would generate the target compound, with concomitant formation of the C3 stereocenter. Successful formation of the C3 (*S*)-alcohol late in the synthesis would obviate potential pitfalls in the construction of the C6-C8 stereotriad.

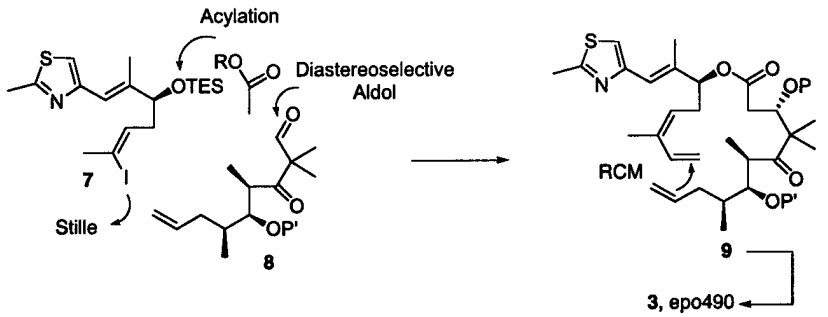
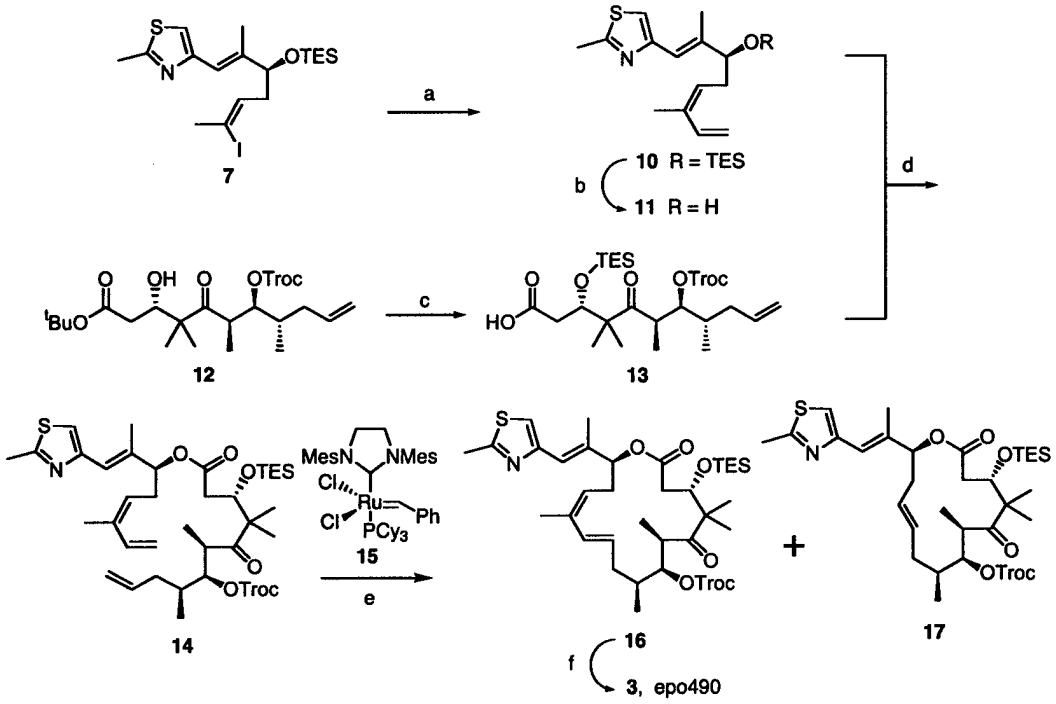


Figure 3. Synthetic plan for epothilone 490.

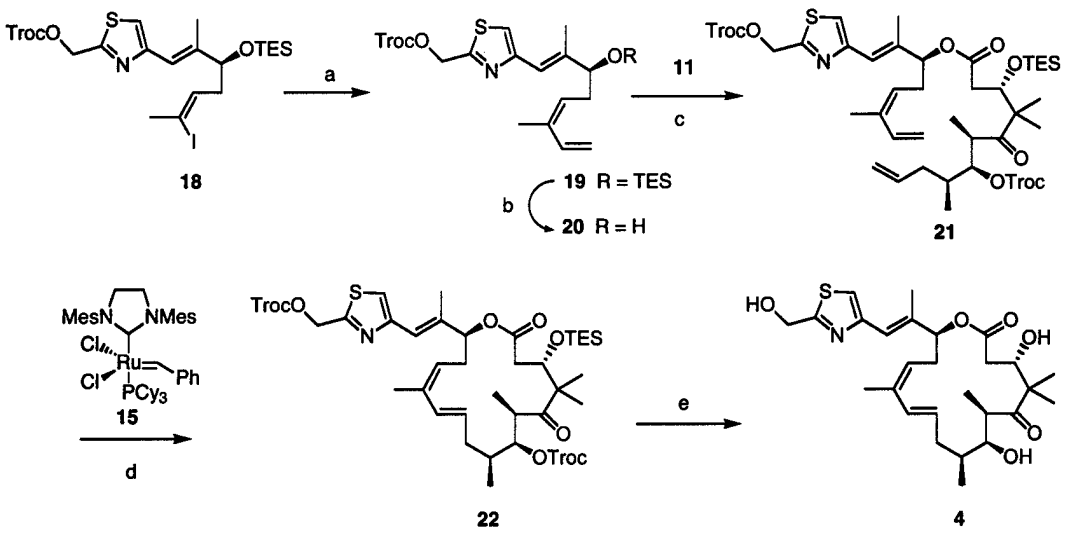
Stille coupling[14] of **7** with vinyl *n*-tributyltin afforded **10** (Scheme 1). Cleavage of the silyl protecting group afforded **11**. Our initial approach commenced with EDCI/DMAP mediated esterification of the resulting allylic alcohol **11** with the C1 acid fragment **13**, obtained

by deprotection of known *tert*-butyl ester **12**.[13] This reaction yielded the cyclization precursor, triene **14**. Exposure of **14** to the ring-closing metathesis reaction with ruthenium metathesis catalyst **15**[15] in methylene chloride gave a mixture of two compounds in a 3:1 ratio, with a total yield of 50%. The major component of the product mixture was identified as the desired *trans*-substituted diene product **16**, along with the 14-membered macrolide **17** as a minor product, seemingly arising from a metathesis reaction involving the internal 12,13-olefin. Removal of the Troc and silyl protecting groups *led to fully synthetic epo490 (3), identical in all respects to an authentic sample*. The formation of the *E*-10,11-double bond was highly stereoselective and helped to confirm the stereochemistry of epo490 to be as shown.



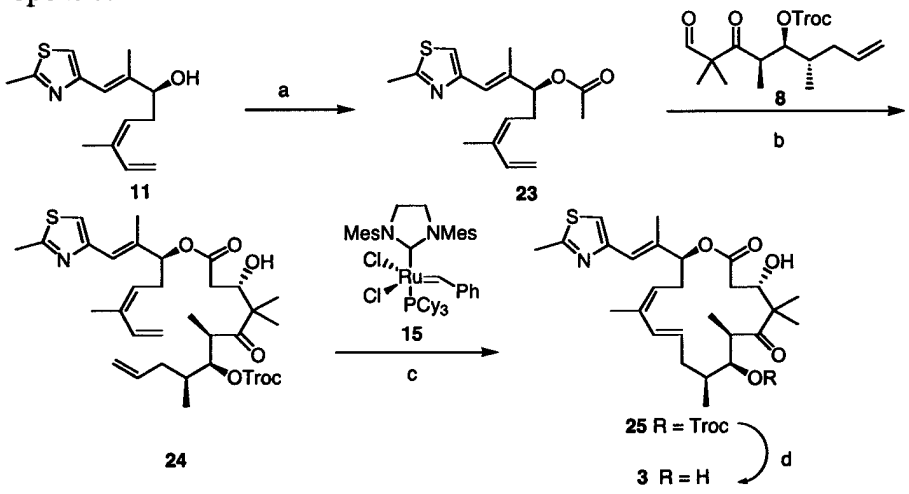
Scheme 1. Initial Ring-Closing Metathesis Route to epo490. Reagents and conditions: (a) $Pd_2(dba)_3$, CH_2=$CHSnBu_3$, PPh_3, DMF, 50 °C, 96%; (b) TBAF, THF, 0 °C, 92%; (c) TESOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to rt, 92%; (d) EDCI, DMAP, CH_2Cl_2, 0 °C to rt, 76%; (e) **15** (10 mol%), CH_2Cl_2 (0.002 M), 35 °C, 50% (**16**:**17** 3:1); (f) Zn, THF, AcOH, 86%; HF·pyr, THF, 0 °C, 90%.

2. Synthesis of 10,11-dehydro-dEpoF (4). Following a similar series of reactions, we synthesized the 21-hydroxyl variant of epo490, 10,11-dehydro-dEpoF, (**4**). Starting with the known Troc-protected 21-hydroxy vinyl iodide **18**,[16] Stille coupling gave diene **19**. Deprotection of the silyl group followed by esterification and ring-closing metathesis afforded **22** (Scheme 2). Deprotection of the Troc and triethylsilyl groups in the usual way afforded diene **4**.



Scheme 2. Synthesis of Compound **4**. Reagents and conditions: (a) $Pd_2(dba)_3$, CH_2=$CHSnBu_3$, PPh_3, DMF, 78%; (b) AcOH, THF, H_2O, 89%; (c) EDCI, DMAP, CH_2Cl_2, 0 °C to rt, 88%; (d) **15** (10 mol%), CH_2Cl_2 (0.002 M), 35 °C, 40%; (e) Zn, THF, AcOH, 70%; HF·pyr, THF, 0 °C, 80%.

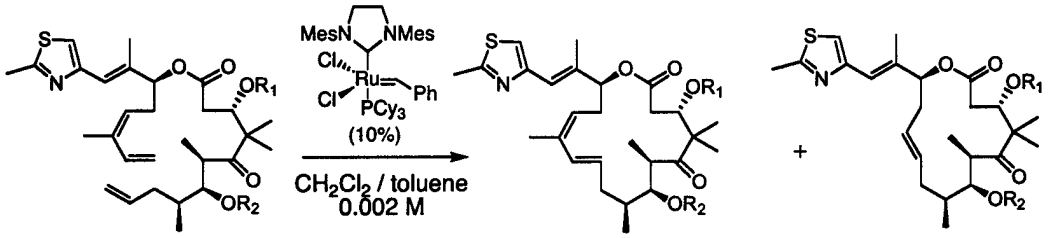
3. Improved synthesis of epo490. Although our initial foray into a RCM manifold afforded only a moderate yield of epo490, we were pleased to observe just the desired olefin in the reaction mixture. Examination of the sequence that led to its construction suggested a different order of joining the fragments in fewer total steps. Since the C3 stereocenter is constructed by a chiral titanium-mediated aldol reaction,[17] we decided to attempt this reaction at a late stage, *with the entire O-alkyl fragment serving as part of the chiral nucleophile as the C15 acetate.* First, alcohol **11** was acylated to obtain the desired acetate **23** (Scheme 3). The lithium enolate of **23** was treated with the chiral titanium reagent to generate the chiral titanium enolate. Addition of aldehyde **8** afforded the desired aldol product, **24**, *as a single diastereomer.* Being aware of the fact that the new ruthenium metathesis catalysts are tolerant of a wider variety of functional groups, we decided to attempt a RCM reaction on **22**, without protection of the C3 alcohol. Treatment of **22** with metathesis catalyst **15** afforded the desired product in 41% yield, *with none of the 14-membered macrolide being observed.* Deprotection of the C7 Troc group in the usual way afforded epo490.



Scheme 3. Epo490 Synthesis via a Late Diastereoselective Aldol Reaction. Reagents and conditions: (a) Ac_2O, DMAP, Et_3N, CH_2Cl_2, 98%; (b) LDA, Et_2O, -78 °C, then $CpTiCl(OR)_2$ (R = 1,2:5,6-di-*O*-isopropylidine-α-L-glucofuranos-3-*O*-yl), -78 °C to -30 °C, then **8**, -78°C, 85%; (c) **15** (10 mol%), CH_2Cl_2 (0.002 M), 35 °C, 41%; (d) Zn, THF, AcOH, 86%.

The change in ratios of the 16- and 14-membered macrolide rings upon deprotection of the C3 alcohol suggested a surprising substrate effect on the macrocyclization step. We began to wonder about the potential effect of deblocking the C7 alcohol on the metathesis reaction as well. Therefore, we decided to perform a series of RCM reactions in which we varied the protection status of the C3 and the C7 alcohols in all the possible combinations (Table 1). The results were indeed quite dependent on the presence of the protecting groups. The 14-membered macrolide was observed only when the substrate was fully protected. More importantly, *the yield of the reaction almost doubled upon use of a substrate where C7 is free.* In fact, RCM of the fully deprotected substrate afforded the product epothilone 490 in 64% yield, with no observed *Z*-isomer of the C10-C11 olefin. This reaction represents the best yield obtained to date in construction of the epothilone scaffold in the B series with RCM, with no laborious separation of undesired olefin isomers involved in the purification process. Interestingly, when we carried out this same series of reactions in refluxing toluene, this substrate effect was diminished, with 55-58% yields observed across the various substrates.

Table 1. Effect of Alcohol Protection and Different Solvents on RCM Yield[a]



14, R_1 = TES, R_2 = Troc	35% / 58%[b]	15% / 6%[b]
24, R_1 = H, R_2 = Troc	41% / 57%	0% / 0%
26, R_1 = TES, R_2 = H	57% / n.d.[c]	0% / n.d.[c]
27, R_1 = H, R_2 = H	64% / 55%	0% / 0%

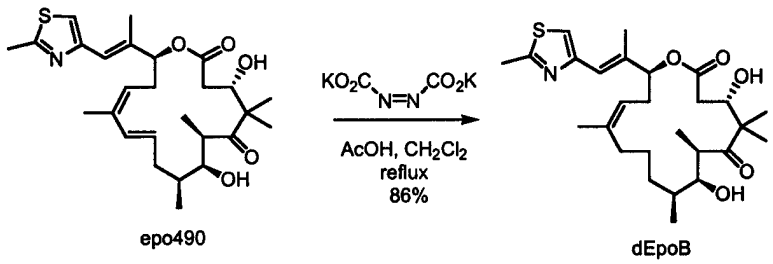
[a]Reactions in CH_2Cl_2 were run for 5.5 h at 35 °C, reactions in toluene for 25 min at 110 °C. [b]Done with 20 mol% catalyst at 0.0005 M dilution. [c]not determined.

The origin of this substrate effect has not yet been determined.[18] Intriguingly, we note that both the C3 and the C7 alcohols are β- to carbonyl groups, suggesting a possible contribution of intramolecular hydrogen bonding in imparting a degree of rigidity to the cyclization precursor. Clearly, this effect does not seem to effect the reaction yield at higher temperature, in a less polar solvent.

4. Practical synthesis of dEpoB – reduction of new olefin in epo490.

The successful application of RCM to the synthesis of the diene epothilones of the 490 series led us to examine if we could access our clinical candidate dEpoB by this newly described endgame. Attainment of this goal would involve a selective hydrogenation of the disubstituted

C10-C11 *E*-olefin, in the presence of the trisubstituted C12-C13 *Z*-olefin and the "benzylic" trisubstituted C16-C17 olefin. A variety of metal-catalyzed and homogeneous hydrogenation conditions were examined, but they suffered from either over- or under-reduction. Diimide-based reductions are known to be extremely sensitive to steric effects in distinguishing differentially substituted olefins.[19] Therefore, we turned our attention to diimide as a reducing agent to convert epothilone 490 to dEpoB. This goal was successfully accomplished by treatment of fully synthetic **3** with *in situ* generated diimide (86% yield, Scheme 4).

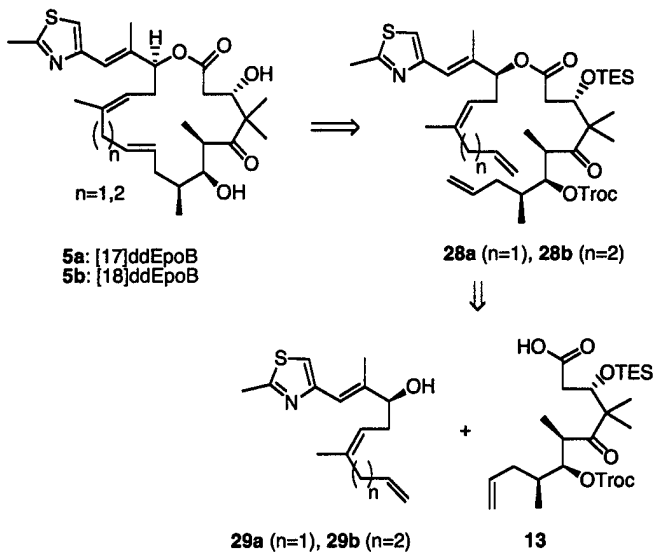


Scheme 4. Diimide Reduction of 10,11-Olefin - New Synthesis of dEpoB.

5. Ring-expanded epothilones: 17- and 18-membered rings.

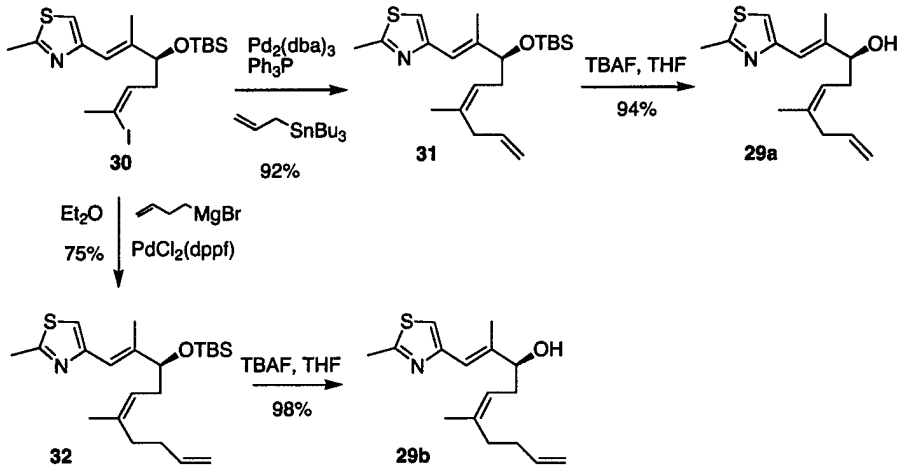
While chemical modifications have been reported for many positions on the epothilone macrolide framework, the effects of ring size with respect to cytotoxic activity have been only briefly studied. The syntheses of 14-, 15-, 17- and 18-membered ring analogs of epothilone A have been reported by Nicolaou and coworkers.[20] These analogs, with the exception of one, had relatively weak tubulin binding activity in comparison to the 16-membered epothilone A (Figure 1; epo A lacks the methyl group at C12, making it a less active congener biologically). The only exception was the [18]desoxyepothilone A, which revealed slightly lower tubulin polymerization activity in comparison to epothilone A. To provide further insight into the correlation between ring size of the epothilones and their corresponding biological activity, especially in the more active epothilone B series, we elected to synthesize the corresponding 17- and 18-membered ring homologs of epo490 (**5a** and **5b**, Figure 2) and evaluate their antitumor activity.

A highly convergent strategy, related to that employed in the synthesis of epo490, was used. Accordingly, fragments of similar complexity served as key building blocks (Scheme 5). We envisioned that the acyl sector **13**, could serve as the polypropionate domain and the alkyl sector **29a** or **29b** would be prepared in a few steps from a known intermediate. The union of the two fragments **29a**(**29b**) and **13** would be initiated through an esterification and consummated via a subsequent ring-closing metathesis. Finally, cleavage of the protecting groups would provide the desired 17- and 18-membered ring homologs (**5a** and **5b**) of epo490.



Scheme 5. Application of RCM Strategy towards the Synthesis of [17]- and [18]ddEpoB.

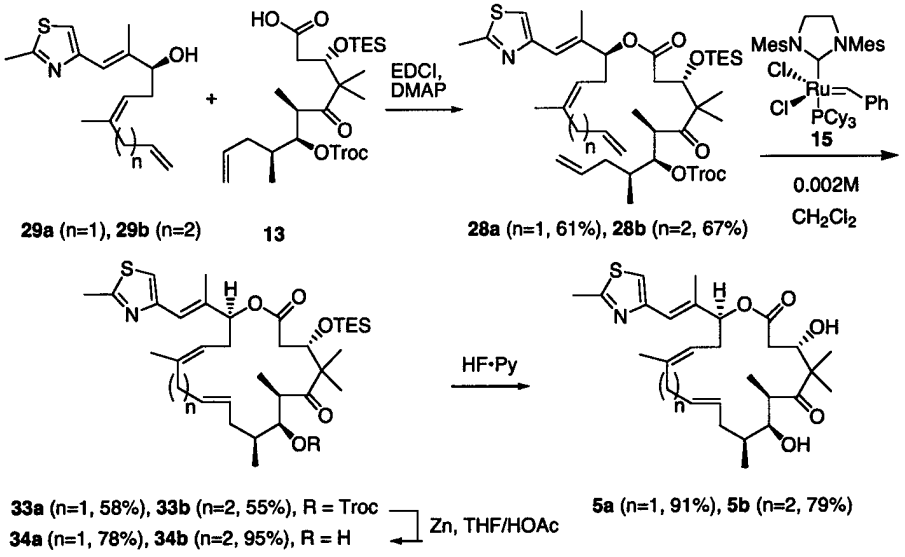
The synthesis of the 17- and 18-membered ring homologs commenced with the conversion of vinyl iodide **30** (available by same sequence as the corresponding TES-protected vinyl iodide **7**, Scheme 1) to the corresponding 1,4-diene **29a** and 1,5-diene **29b** (Scheme 6). Reaction of **30**, with allyltributyltin under Stille conditions, afforded the desired 1,4-diene **31** in 92% yield. Correspondingly, reaction with butenylmagnesium bromide under the Tamao-Kumada-Corriu palladium(0)-mediated coupling conditions[21] provided the desired 1,5-diene **32** in 75% yield. It seems likely that this reaction could be extended towards the synthesis of alternative unconjugated dienes, which could allow for the synthesis of even larger ring analogs. Finally, treatment of 1,4-diene **31** and 1,5-diene **32** with tetra-*n*-butylammonium fluoride accomplished the deprotection of the secondary alcohols in high yield.



Scheme 6. Preparation of fragments **29a** and **29b**.

Esterification of the resultant allylic alcohols **29a** and **29b** with acid **13** provided the corresponding cyclization precursors in 61% (**28a**) and 67% (**28b**) yields, respectively (Scheme 7). The RCM reaction of **28a** was then carried out using the catalyst **15**, which provided, as in our earlier study with epo490, exclusively the *trans* isomer **33a** in a yield of 58%. Using the

same RCM reaction conditions with the **28b** also provided exclusively the *trans* isomer **33b** in 55% yield. Finally, cleavage of the Troc protecting group followed by deprotection of triethylsilyl ether with hydrogen fluoride-pyridine led to **5a** and **5b**.

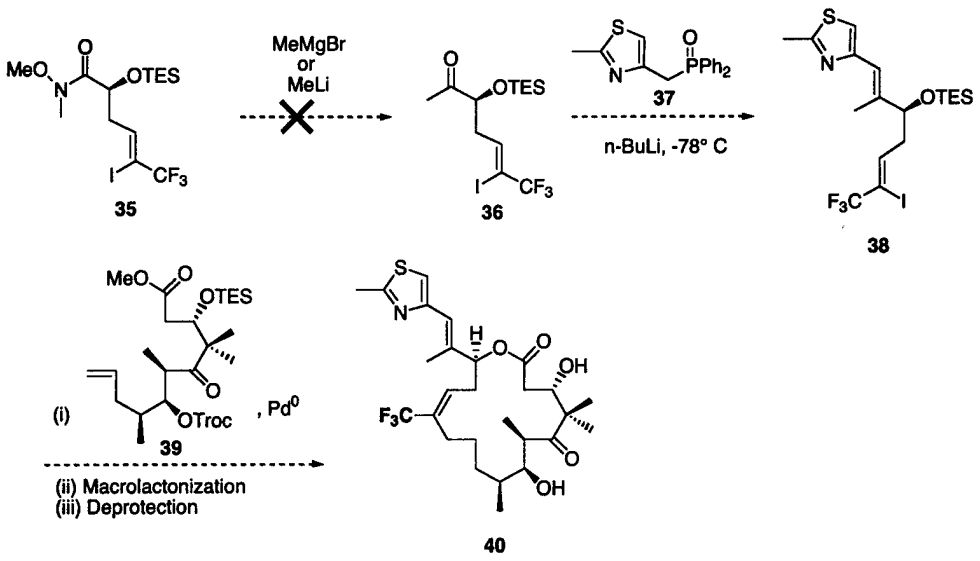


Scheme 7. Completion of the syntheses of [17]ddEpoB and [18]ddEpoB.

6. Introduction of a trifluoromethyl substitution in the epothilone framework.

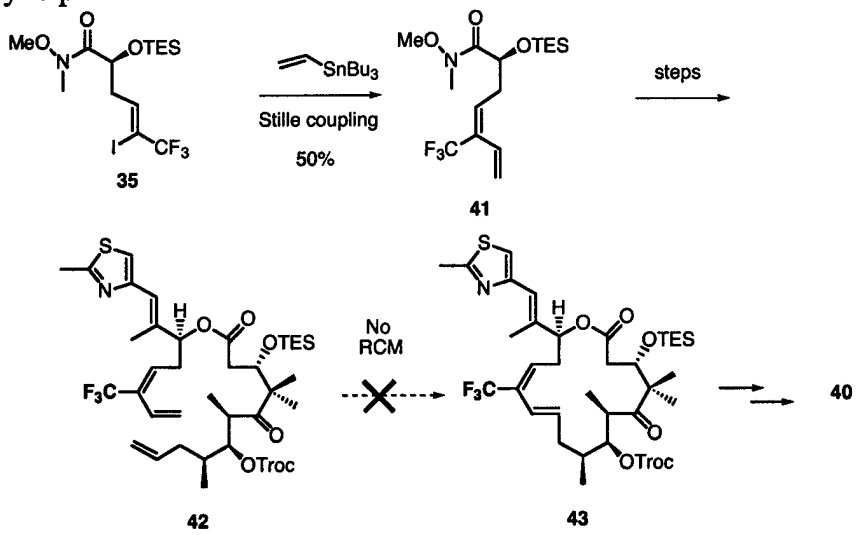
In the hopes of improving the therapeutic profile of (dEpoB, **1**), we opted to explore the effect of the trifluoro substitution at the C-26 position. The incorporation of fluorine in the related taxoid drugs has been shown by Ojima and coworkers to improve their potency.[22] Recently, a new fluorinated epothilone analog, the 26-fluoroepothilone B, has been reported to have a wider therapeutic index than epothilone B,[23] which set precedence for our efforts.

Our initial goal was to synthesize 10,11-dehydro-12,13-desoxy-26-trifluoro-epothilone B ($26CF_3$-[16]ddEpoB, **40**, Scheme 8) using our old *B*-alkyl Suzuki – macrolactonization strategy used for the synthesis of dEpoB.[24] Although we were able to synthesize the Weinreb's amide **35** (*vide infra*), further transformations to access the final product was thwarted by the inability to install the methyl ketone in **36** by any known method. This failure was ascribed to the extreme reactivity of the vinyl iodide (α- to the very electron withdrawing trifluoro group) to the basic reaction conditions and subsequent decomposition.



Scheme 8. Proposed (unsuccessful) scheme for synthesis of 26-trifluoro-dEpoB (**40**).

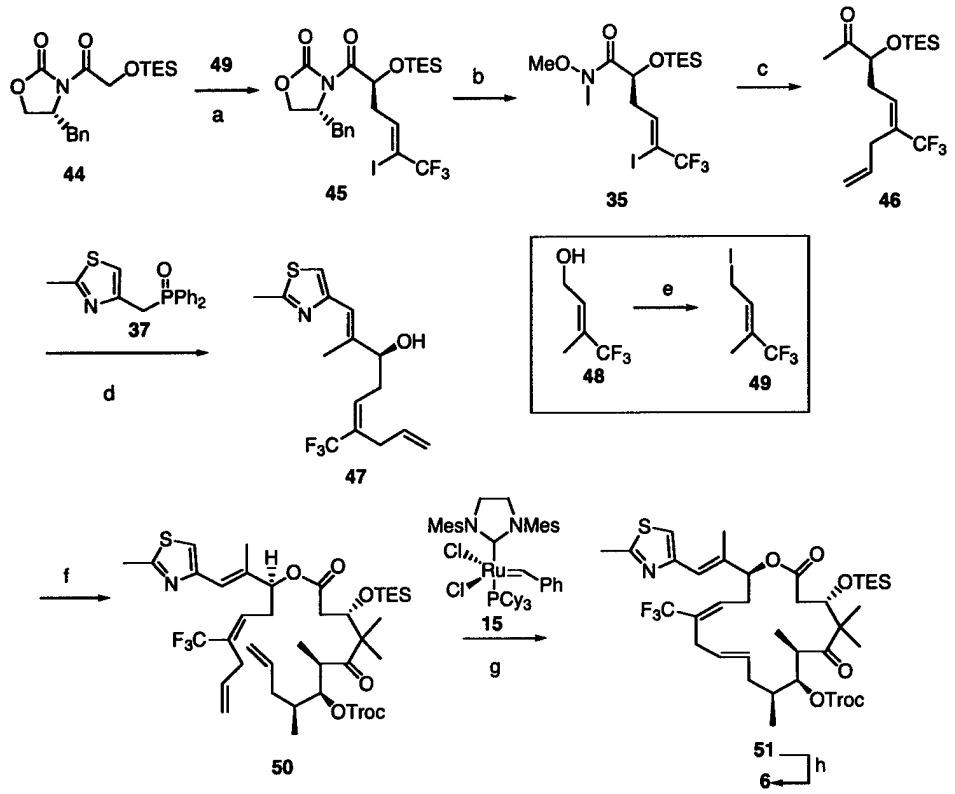
Upon successful optimization of our new RCM-based strategy for assembling the epothilones, we attempted to apply the lessons learnt in that campaign to the synthesis of the 26-CF_3 epothilones. Since we introduce a 2-carbon spacer as a vinyl group at the offending vinylic carbon by Stille coupling, we assumed that the extra reactivity of the CF_3-substituted vinyl iodide could be attenuated by early conversion to the 1,3-diene ultimately required for olefin metathesis. In this regard, the vinyl iodide **35** was converted to the 1,3-diene **41** and subsequently transformed into the RCM precursor **42** (Scheme 9). However, subjection of **42** to olefin metathesis, under a variety of conditions, did not afford any ring-closed product **43**. This is also perhaps due to the electron-deficient nature of the reacting olefin, substituted by the CF_3-group at the allylic position.



Scheme 9. Failed RCM route to 26-CF_3-dEpoB.

We reasoned that the negative effect of the 26-trifluoro substitution on the RCM reaction might be alleviated by adding a carbon spacer between the RCM reaction center and the trifluoromethyl group. This line of reasoning was also prompted by the successful synthesis and

encouraging *in vitro* cytotoxicity (*vide infra*) of [17]ddEpoB (**5a**). Accordingly, we undertook a synthesis of 10,11-dehydro-13,14-desoxy-27-trifluoro-[17]epothilone B (27-F_3-[17]ddEpoB, **6**, Figure 2) via the ring-closing metathesis of **50**, which contained the 1,4-diene required to accommodate the new goal (Scheme 10). A comparison of the biological profile of 27-F_3-[17]ddEpoB (**6**) with that of **5a** would allow us to evaluate the effect of the trifluoromethyl substitution. The synthesis of **6** commenced by the preparation of the alkyl sector **47**. Alkylation of the previously reported lithium enolate of **44**[24] with allyl iodide **49** afforded compound **45** in 78% yield and high diastereoselectivity (>25:1 de). Allyl iodide **49** was synthesized from known alcohol **48**[25] using TMSI in methylene chloride. Compound **45** was then advanced in three steps to the previously mentioned Weinreb amide **35**. Reaction of **35** with allyltributyltin under Stille conditions followed by methyl Grignard addition gave the desired ketone **46**. Wittig condensation of ketone **46** with known phosphine oxide **37** followed by deprotection of the triethylsilyl ether gave the alkyl fragment **47** in good yield. Esterification of **47** with C1-C10 acid fragment **13** (see Scheme 1) provided the RCM precursor **50** in 75% yield. The RCM reaction of **50** was then carried out using the catalyst **15** in methylene chloride, this time successfully providing exclusively the *trans* isomer **51** in 57% yield. Finally, reductive cleavage of the Troc protecting group with zinc and acetic acid, followed by deprotection of the TES ether with HF-pyridine, provided 27-F_3-[17]ddEpoB (**6**).



Scheme 10. Synthesis of 10,11-dehydro-13,14-desoxy-27-trifluoro-[17]epothilone B. Reagents and conditions: (a) LiHMDS, **49**, -78 °C, 78%; (b) i) HOAc:THF:H_2O (3:1:1); ii) CH_3ONHCH_3, $AlMe_3$; iii) TESCl, imidazole, DMF, 79% overall; (c) i) Allyltributyltin, Pd_2dba_3, PPh_3, DMF, 50 °C, 3h, 74%; ii) MeMgBr, THF, 0 °C, 93%; (d) i) **37**, *n*-BuLi, THF, -78 °C, 30 min., ii) **46**, -78 °C to rt, 85%; iii) HOAc:THF:H_2O (3:1:1), 98%; (e) TMSI, CH_2Cl_2, 0 °C, 92%; (f) EDCI, DMAP, **13**, 75%; (g) **15** (10 mol%), CH_2Cl_2, 57%; (h) Zn, AcOH, THF; HF-pyr, THF, 0 °C, 73% (2 steps).

7. Anticancer evaluation of new epothilones.

With the successful syntheses of these new epothilone analogues, we were well positioned to investigate the *in vitro* cytotoxicities of these compounds in cell culture assays. One of the significant advantages that the epothilone family of chemotherapeutic agents possesses over traditional oncolytics like taxol, adriamycin, vinblastine, vincristine, etoposide etc. is the lack of susceptibility to multi-drug resistance (MDR). We, therefore, also examined the newly synthesized analogues for the concentrations required for 50% growth inhibition (IC_{50}'s) of a variety of MDR-susceptible cancer (leukemia) cell lines.[9] Since the mechanism of action of the epothilones involve the stabilization of microtubule assembly, we examined the relative stabilization of microtubules by these compounds when compared to our drug candidate dEpoB (**1**).

Table 2. *In vitro* Cytotoxicities (IC_{50}) with tumor cell lines[a] and Microtubule Binding[b].

Compound	CCRF-CEM (μM)	CCRF-CEM/$_{VBL100}$ (μM)	CCRF-CEF/$_{VM1}$ (μM)	CCRF-CEM/$_{Taxol}$ (μM)	% Tubulin Binding[b]
1 (dEpoB)	0.011	0.015	0.016	0.007	100
3	0.025	0.091	0.035	0.032	89
4	0.030	0.202	0.061	0.051	77
5a	0.040	0.126	0.055	0.053	94
5b	0.322	0.87	n.d.[c]	0.508	51
6	0.068	0.191	n.d.[c]	n.d.[c]	n.d.[c]
Taxol	0.0021	0.827	0.003	0.081	n.d.[c]
Vinblastine	0.0008	0.122	0.0014	0.018	n.d.[c]

[a]XTT assay following 72 h inhibition. CCRF-CEM is a human T-cell acute lymphoblastic leukemia cell line. The CCRF-CEM/$_{VBL100}$, CCRF-CEM/$_{VM1}$ and CCRF- CEM/$_{Taxol}$ cell lines all overexpress P-glycoprotein and display a multidrug resistance phenotype to MDR associated oncolytics.[9] [b]Formation of microtubules in the presence of the compounds. Microtubules formed in the presence of dEpoB is defined as 100%. [c]not determined.

As seen in Table 2, the newly synthesized analogues have cytotxicities comparable to dEpoB, except the 18-member ring-expanded epothilone **5b**. They also maintain the MDR property of the parent epothilones. Comparison of **5a** and **6** shows that there is no significant effect of a CF_3 substitution at the C27 position, at least in the 17-membered homolog series. Study of the corresponding effect in the 16-membered parent series awaits successful synthesis of 26-CF_3-dEpoB. Encouragingly, the microtubule stabilizing ability of the newly synthesized analogues closely parallels the observed cytotoxicity data, suggesting a similar mechanism of action.

The impressive cell growth inhibition exhibited by epo490 across a range of various drug-resistant tumors led us to determine its efficacy in an *in vivo* setting, in nude mice bearing human tumor xenografts. Fortunately, our straightforward synthesis of **3** allowed us to indulge these interests. To our surprise, epo490 did not demonstrate any meaningful inhibitory effect on

the growth of the implanted tumors, as compared to control mice (data not shown). These data were surprising given the favorable prognosis based on *in vitro* protocols.

In addressing this problem, we recalled that dEpoB itself evidenced a worrisome bioinstability in murine plasma. However, it had much longer half-lives in higher organisms, including humans.[9] This trend has been ascribed to higher esterase levels in rodents. The failure of epo490 in our murine xenograft assay, in contrast to its excellent cell-culture inhibitory data, suggested that the pharmacokinetic properties of **3** be evaluated.

Indeed, on exposure of **1** and **3** to murine plasma, the biodegradation of epothilone 490 was even faster than that of dEpoB (Figure 4). Thus, while the murine pharmacokinetics of **1** are far from optimal, drug levels are adequate for major reduction and elimination of murine tumor burden. By contrast, the murine stability of **3** is so poor as to vitiate the potential benefits of the drug. *Encouragingly, the same drug remained essentially unchanged over 3 h in human plasma*, suggesting the need for *in vivo* studies in higher mammals for a more effective evaluation as a potential drug candidate.

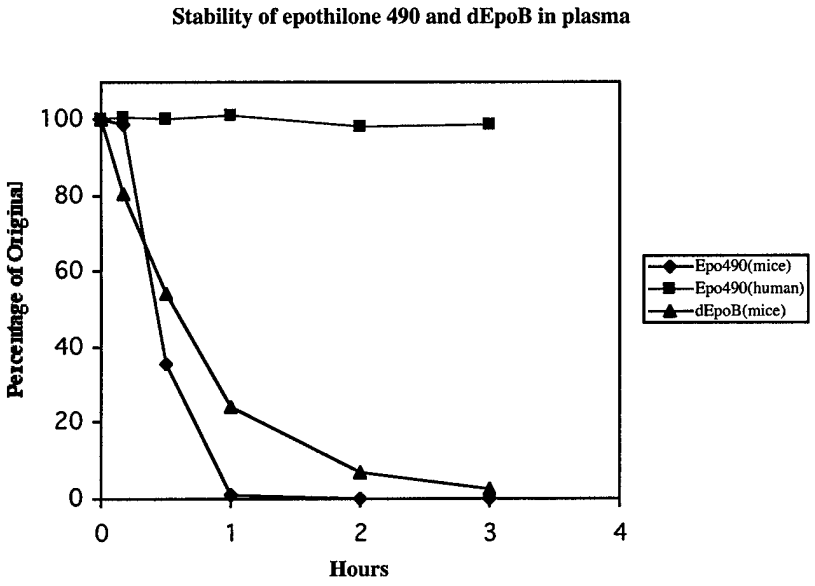


Figure 4. Plasma stability of epothilone 490 and dEpoB in nude mouse and human plasma (see Ref. 9 for details).

KEY RESEARCH ACCOMPLISHMENTS

- First synthesis of 10,11-dehydro-epothilones
- New, more efficient synthesis of clinical candidate 12,13-desoxyepothilone B
- Examination of ring-size to anticancer ability by synthesis of 17- and18-membered epothilones
- First synthesis of a CF_3-susbtituted epothilone
- Evaluation of *in vitro* cytotoxicities and microtubule stabilization ability of new analogues
- Pharmacokinetic study of 10,11-dehydropeothilone B (epo490) in murine and human plasma

REPORTABLE OUTCOMES

Publications:

1. Stachel, S.J.; **Biswas, K.**; Danishefsky, S.J. "Epothilones, Eleutherobins, and Related Types of Molecules" *Curr. Pharm. Design* **2001**, *7*, 1277-1290.

2. **Biswas, K.**; Lin, H.; Njardarson, J.T.; Chappell, M.D.; Chou, T.C.; Guan, Y.; Tong, W.P.; He, L.; Horwitz, S.B.; Danishefsky, S.J. "Highly Concise Routes to Epothilones: The Total Synthesis and Evaluation of Epothilone 490" *J. Am. Chem. Soc.* **2002**, *124*, 9825-9832.

3. Rivkin, A.; Njardarson, J.T.; **Biswas, K.**; Chou, T.C.; Danishefsky, S.J. "Total Syntheses of [17]- and [18]-Dehydrodesoxyepothilones B via a Concise Ring-Closing Metathesis-Based Strategy: Investigation of Ring Size with Biological Activity" *J. Org. Chem.* (in press).

4. Rivkin, A.; **Biswas, K.**; Chou, T.C.; Danishefsky, S.J. "On the Introduction of a Trifluoromethyl Substituent in the Epothilone Setting: Chemical Issues Related to Ring Forming Olefin Metathesis and Earliest Biological Findings" *Org. Lett.* (submitted).

Patents:

1. "Synthesis of Epothilones, Intermediates Thereto and Analogues Thereof" Danishefsky, S.J.; **Biswas, K.**; Chappell, M.D.; Lin, H.; Njardarson, J.T.; Lee, C.; Chou, T.-C. US Provisional Patent Application 60/317,637, filed September 6, 2001.

Presentations:

1. "Molecular Approaches to Cancer Therapy," Seminar, Kosan Biosciences, Hayward, CA (Nov. 2001).

2. "Molecular Approaches to Cancer Therapy," Seminar, Tularik, South San Francisco, CA (Nov. 2001).

3. "Molecular Approaches to Cancer Therapy," Seminar, Amgen, Thousand Oaks, CA (Dec. 2001).

4. "Molecular Approaches to Cancer Therapy," Seminar, Merck Research Laboratories, San Diego, CA (Jan. 2002).

5. Chou, T.C.; Guan, Y.; **Biswas, K.**; Chappell, M.D.; Lin, H.; Danishefsky, S.J. "Antitumor Efficacy Determinants of Epothilones" Poster 3922, American Association for Cancer Research annual meeting, San Francisco, CA (Apr. 2002).

Employment:
Appointment as Research Scientist I, Department of Medicinal Chemistry, Amgen, Thousand Oaks, CA.

CONCLUSIONS

We have achieved the chemical synthesis and biological evaluation of various natural and designed epothilone-based potential chemotherapeutic agents. Our earlier studies led to the development of 12,13-desoxyepothilone B (dEpoB) as a clinical candidate for solid tumors, currently being evaluated at UCLA and Memorial Sloan-Kettering Cancer Center. We continue to seek good back-up candidates for our clinical progam, and have therefore synthesized and evaluated new epothilones in this study.

Herein, we described a construction of the epothilones with ring-closing metathesis. For purposes of greater synthetic convergency, we fashioned the C3-(*S*)-alcohol late in the synthesis, using a chiral titanium-mediated aldol reaction with the entire *O*-alkyl fragment as its C15 acetate as the enolate component. Examination of the effects of protecting groups on the RCM process demonstrated an intriguing substrate effect on reaction yield. Selective diimide reduction of the new olefin yielded dEpoB, our current clinical candidate, *validating the utility of this new RCM-reduction protocol in efficiently generating the epothilone framework.* The ability of this new route to deliver other dehydroepothilones, including ring-expanded congeners are described. We have also undertaken the first examination of the effect of a trifluoromethyl substitution on epothilone activity. These compounds show similar cytotoxicities and microtubule stabilization capabilities when compared to dEpoB. Ring-expansion strategies afford similar activity upto the 17-membered congener, with a drop in efficacy being observed in the 18-membered dehydroepothilone.

Also described is the surprisingly poor *in vivo* performance of epo490 in xenografts. This outcome was traceable to unfavorable pharmacokinetic features of the drug in this particular specie. *To the extent that plasma stability is predictive of pharmacokinetic performance, the prognosis for the effectiveness of* ***3*** *in humans is much more promising.* Parenthetically, this research points to exciting possibilities in drug discovery and refinement centered around organic synthesis in close liaison with *in vivo* pharmacology and pharmacokinetics.

REFERENCES

[1] Horwitz, S. B.; Fant, J.; Schiff, P. B. *Nature* **1979**, *277*, 665.

[2] Taxol is a registered trademark of Bristol-Myers Squibb. Nicolaou, K. C.; Dai, W .-M.; Guy, R. K. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 15.

[3] Gerth, K.; Bedorf, N.; Hofle, G.; Irschik, H.; Reichenbach, H. *J. Antibiot.* **1996**, *49*, 560.

[4] Bollag, D. M.; McQueney, P. A.; Zhu, J.; Hensens, O.; Koupal, L.; Liesch, J.; Goetz, M.; Lazarides, E.; Woods, C. M. *Cancer Res.* **1995**, *55*, 2325.

[5] Kowalski, R. J.; Terhaar, E.; Longley, R. E.; Gunasekera, S. P.; Lin, C. M.; Day, B. V.; Hamel, E. *Mol. Biol. Cell* **1995**, *6*, 2137.

[6] (a) Meng, D.; Bertinato, P.; Balog, A.; Su, D .-S.; Kamenecka, T.; Sorensen, E. J.; Danishefsky, S. J . *J. Am. Chem. Soc.* **1997**, *119*, 10073. (b) Balog, A.; Meng, D.; Kameneka, T.; Bertinato, P.; Su, D.-S.; Sorensen, E. J.; Danishefsky, S. J. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2801.

[7] (a) Nicolaou, K. C.; He, Y.; Vourloumis, D.; Vallberg, H.; Yang, Z. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2014 and references therein. For recent chemical syntheses of epothilone B. see: (b) Sawada, D.; Kanai, M.; Shibasaki, M. *J. Am. Chem. Soc.* **2000**, *122*, 10521. (c) Mulzer, J.; Mantoulidis, A.; Ohler, E. *J. Org. Chem.* **2000**, *65*, 7456. (d) White, J. D.; Carter, R. G.; Sundermann, K. F. *J. Org. Chem.* **1999**, *64*, 684. (e) May, S. A.; Grieco, P. A. *Chem. Comm.* **1998**, 1597.

[8] (a) Chou, T. C.; Zhang, X. G.; Harris, C. R.; Kuduk, S. D.; Balog, A.; Savin, K. A.; Bertino, J. R.; Danishefky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15798; (b) Chou, T. C.; Zhang, X. G.; Balog, A.; Su, D.; Meng, D. F.; Savin, K.; Bertino, J. R.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9642.

[9] Chou, T. C.; O'Connor, O. A.; Tong, W. P.; Guan, Y.; Zhang, Z .-G.; Stachel, S. J.; Lee, C.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8113.

[10] Arslanian, R. L.; Tang, L.; Blough, S.; Ma, W.; Qiu, R. -G.; Katz, L.; Carney, J. R. *J. Nat. Prod.* **2002**, *65*, 1061.

[11] Reviews: (a) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* **1995**, *28*, 446; (b) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18; (c) *Alkene Metathesis in Organic Chemistry* Ed.: Fürstner, A.; Springer, Berlin, **1998**; (d) Fürstner, A. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 3012; (e) Schrock, R. R. *Top. Organomet. Chem.* **1998**, *1*, 1.

[12] Chappell, M. D.; Stachel, S. J.; Lee, C. B.; Danishefsky, S. J. *Org. Lett.* **2000**, *2*, 1633.

[13] Wu, Z.; Zhang, F.; Danishefsky, S. J. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 4505.

[14] Farina, V.; Krishnamurthy, V.; Scott, W. J. *Org. React.* **1997**, *50*, 1.

[15] Scholl, M.; Trnka, T. M.; Morgan, J. P.; Grubbs, R. H. *Tetrahedron Lett.* **1999**, *40*, 2247.

[16] Lee, C. B.; Chou, T .-C.; Zhang, X. G.; Wang, Z. G.; Kuduk, S. D.; Chappell, M. D.; Stachel, S. J.; Danishefsky, S. J. *J. Org. Chem.* **2000**, *65*, 6525.

[17] Duthaler, R. O.; Herold, P.; Lottenbach, W.; Oretle, K.; Reidiker, M. *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 495.

[18] For examples and discussion of similar protecting group effects on RCM reactions in the synthesis of salicylihalamides, see: (a) Fürstner, A.; Thiel, O.; Blanda, G. *Org. Lett.* **2000**, *2*, 3731; (b) Fürstner, A.; Dierkes, T.; Thiel, O.; Blanda, G. *Chem. Eur. J.* **2001**, *7*, 5286, and references therein.

[19] (a) Corey, E. J.; Mock, W. L.; Pasto, D. J. *Tetrahedron Lett.* **1961**, 347; (b) Pasto, D. J.; Taylor, R. T. *Org. React.* **1991**, *40*, 91. Importantly, J.D. White and coworkers have previously reported the reduction of a C9-C10 olefin during their synthesis of the epothilones with diimide, see Ref. 7d.

[20] Nicolaou, K. C.; Sarabia, F.; Ninkovic, S.; Ray, M., Finlay, V., Boddy, C. N. C. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 81.

[21] (a) Tamao, K., Sumitani, K., Kumada, M. *J. Am. Chem. Soc.* **1972**, *94*, 4374; (b) Corriu, R. J., Masse, J. P. *Chem. Comm.* **1972**, 144.

[22] Ojima, I.; Lin, S. N.; Slater, J. C.; Wang, T.; Pera, P.; Bernacki, R. J.; Ferlini, C.; Scrambia, G. *Bioorg. Med. Chem.* **2000**, *8*, 1619.

[23] Newman, R. A.; Yang, J.; Raymond, M.; Finlay, V.; Cabral, F.; Vourloumis, D.; Stephens, L. C.; Troncoso, P.; Wu, X.; Logothetis, C. J.; Nicolaou, K. C.; Navone, N. M. *Cancer Chemother. Pharmacol.* **2001**, *48*, 319.

[24] Lee, C. B.; Wu, Z.; Zhang, F.; Chappell, M. D.; Stachel, S. J.; Chou, T. C.; Guan, Y.; Danishefksy, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 5249.

[25] Obtained by HI addition to the commercially available trifluoromethyl alkynoate and DIBAL-H reduction.

APPENDICES

- Curriculum Viate of the principal investigator (2 pages)
- Reprints of published and submitted papers (4 articles)

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EDUCATION

7/00 - present: Post-Doctoral Fellow, Memorial Sloan-Kettering Cancer Center, New York, NY. Advisor: Professor Samuel J. Danishefsky. Synthesized new epothilones for cancer chemotherapy and glycosyl amino acids by olefin metathesis for multi-antigen carbohydrate-based antitumor vaccines.

9/94 – 6/00: Ph.D. in Chemistry, Princeton University, Princeton, NJ. Advisor: Professor Daniel E. Kahne. Synthesized oligosaccharide analogues of anticancer agent calicheamicin and developed new capillary electrophoresis assay for measuring affinity to DNA.

7/92 - 5/94: M.Sc. in Chemistry, Indian Institute of Technology, Kanpur, India. Advisor: Professor Javed Iqbal. GPA 10.0/10.0 (ranked 1st/30).

7/89 - 6/92: B.Sc. (Honours) in Chemistry, St. Stephen's College, University of Delhi, Delhi, India.

AWARDS

- US Army Post-Doctoral Fellowship in Breast Cancer Research (2001).
- Lawrence and Selma Ruben Fellowship in Cancer Research (2001).
- Bristol-Myers Squibb Graduate Fellowship in Synthetic Organic Chemistry (1998).
- General Proficiency Medal for Best Academic Performance in Chemistry, Indian Institute of Technology, Kanpur, India (1994).

PUBLICATIONS

1. Rivkin, A.; **Biswas, K.**; Chou, T.C.; Danishefsky, S.J. "On the Introduction of a Trifluoromethyl Substituent in the Epothilone Setting: Chemical Issues Related to Ring Forming Olefin Metathesis and Earliest Biological Findings" *Org. Lett.* (submitted).

2. Rivkin, A.; Njardarson, J.T.; **Biswas, K.**; Chou, T.C.; Danishefsky, S.J. "Total Syntheses of [17]- and [18]-Dehydrodesoxyepothilones B via a Concise Ring-Closing Metathesis-Based Strategy: Investigation of Ring Size with Biological Activity" *J. Org. Chem.* **2002** (in press).

3. **Biswas, K.**; Coltart, D.M.; Danishefsky, S.J. "Construction of Carbohydrate-Based Antitumor Vaccines: Synthesis of Glycosyl Amino Acids by Olefin Cross-Metathesis" *Tetrahedron Lett.* **2002**, *43*, 6107-6110.

4. **Biswas, K.**; Lin, H.; Njardarson, J.T.; Chappell, M.D.; Chou, T.C.; Guan, Y.; Tong, W.P.; He, L.; Horwitz, S.B.; Danishefsky, S.J. "Highly Concise Routes to Epothilones: The Total Synthesis and Evaluation of Epothilone 490" *J. Am. Chem. Soc.* **2002**, *124*, 9825-9832.

5. Stachel, S.J.; **Biswas, K.**; Danishefsky, S.J. "Epothilones, Eleutherobins, and Related Types of Molecules" *Curr. Pharm. Design* **2001**, *7*, 1277-1290.

6. **Biswas, K.**; Pal, S.; Carbeck, J.D.; Kahne, D. "The Molecular Basis for Pyrimidine Selective DNA Binding: Analysis of Calicheamicin Oligosaccharide Derivatives by Capillary Electrophoresis" *J. Am. Chem. Soc.* **2000**, *122*, 8413-8420.

7. Kalben, A.; Pal, S.; Andreotti, A.H.; Walker, S.; Gange, D.; **Biswas, K.**; Kahne, D. "Calicheamicin-DNA Recognition: An Analysis of Seven Different Drug-DNA Complexes" *J. Am. Chem. Soc.* **2000**, *122*, 8403-8412.

8. Bradley, A.Z.; Link, A.J.; **Biswas, K.**; Kahne, D.; Schwartz, J.; Jones, M.; Zhu, Z.; Platz, M.S. "Hydrogen Abstraction on Photolysis of a Naphthocarborane" *Tetrahedron Lett.* **2000**, *41*, 8691-8694.

9. Liang, R.; Yan, L.; Loebach, J.; Ge, M.; Uozumi, Y.; Sekanina, K.; Horan, N.; Gildersleeve, J.; Thompson, C.; Smith, A.; **Biswas, K.**; Still, W.C.; Kahne, D. "Parallel Synthesis and Screening of a Solid Phase Carbohydrate Library" *Science*, **1996**, *274*, 1520-1522.

PATENT APPLICATIONS

- Danishefsky, S.J.; **Biswas, K.**; Chappell, M.D.; Lin, H.; Njardarson, J.T.; Lee, C; Chou, T.C."Synthesis of Epothilones, Intermediates Thereto and Analogues Thereof" US Provisional Patent Application 60/317,637 filed 09/06/2001.

PRESENTATIONS

- Chou, T.C.; Guan, Y.; **Biswas, K.**; Chappell, M.D.; Lin, H.; Danishefsky, S.J. "Antitumor Efficacy Determinants of Epothilones" Poster 3922, American Association for Cancer Research annual meeting, 4/2002.
- Koide, F.; Ragupathi, G.; Williams, L.J.; **Biswas, K.**; Slovin, S.F.; Danishefsky, S.J.; Livingston, P.O. "Characterization of affinity purified anti-Tn(c) and anti-TF(c) antibodies obtained from prostate cancer patients vaccinated with Tn(c)-KLH or TF(c)-KLH conjugate vaccines" Poster 2781, American Association for Cancer Research annual meeting, 4/2002.
- **Biswas, K.** "Synthesis of Modified Calicheamicin Oligosaccharides as Probes for DNA Recognition". Bristol-Myers Squibb Award Symposium, Wallingford, CT, 3/25/1999.

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The Epothilones, Eleutherobins, and Related Types of Molecules

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Abstract. Taxol® is currently one of the most effective anticancer agents available. However, limitations due to multidrug-resistance (MDR) susceptibility and lack of aqueous solubility render it less than an ideal drug. These limitations, coupled with taxol's unique mechanism of tumor inhibition, involving the stabilization of microtubule assembly, have spurred the search for more effective chemotherapeutic agents. This review will discuss the chemistry and biology of some of the most promising new molecules with "taxol-like" activity. The extended family of microtubule-stabilizing agents now includes the epothilones, eleutherobins, discodermolide, laulimalide and WS9885B. The epothilones have emerged as one of the most exciting new candidates for detailed structure-activity-related studies. A review of our efforts in the synthetic and biological aspects of this research is presented, as are the latest developments reported from other laboratories in academia and the pharmaceutical industry. The synthesis and structure-activity studies of eleutherobins, as well as recent progress with discodermolide, laulimalide and WS9885B are also reviewed. An abundance of exciting advances in chemistry and biology have emerged from these studies, and it is hoped that it will ultimately result in the development of new and more effective chemotherapeutic agents in the fight against cancer.

INTRODUCTION

Chemotherapy, for all its limitations, is one of the most widely practiced methods in the treatment of various cancers. The problems associated with chemical treatment as a means of therapy arises from the necessity to differentiate between normal and aberrant cells of the same genealogical origin. Traditionally, chemotherapeutic agents act by interfering with DNA replication during mitosis [1]. In this process, rapidly dividing cells are most susceptible to cellular disruption, which leads to subsequent cell death. Unfortunately, due to the non-specific nature of the interactions between these drugs and DNA, many normal cells are also adversely affected in this process. This collateral damage leads to side effects and potential harm to the recipient that tempers the effectiveness of treatment. Hence, there still exists a profound need for more effective chemotherapeutic agents.

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Tubulin, the basic subunit of microtubules and one of the most highly conserved proteins in evolution, has emerged as a promising new target for chemotherapeutic intervention. Microtubules are dynamic, polymeric structures that are an integral part of all eukaryotic cells [2], [3]. Perhaps most significantly, they play a crucial role in mitosis (cell division). During mitosis, the dynamics of microtubule polymerization and depolymerization are finely controlled and any variation in the rate of polymerization can profoundly effect cellular replication. Affected cells are unable to pass through a checkpoint in the cell cycle and enter into programmed cell death, a process known as apoptosis. It is by effecting the rate of polymerization/ depolymerization during this critical junction in the cell cycle that a new class of chemotherapeutic agents have emerged [4].

There are two major classes of chemotherapeutic agents that induce mitotic arrest by disrupting microtubule dynamics; those that depolymerize tubulin and those that stabilize tubulin polymers. Depolymerization agents, such as colchicine [5,6], vinblastine [7], podophyllotoxin [8], and vincristine [5,7],operate

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by inhibiting the formation of microtubule spindles or depolymerizing existing ones.

The second class of chemotherapeutic agents, and the focus of this review, operates by initiating tubulin polymerization as well as hyper-stabilizing existing microtubules. The most well known of this class of tubulin stabilizing agents is taxol® (paclitaxel), which is currently a front-line anticancer agent [9].

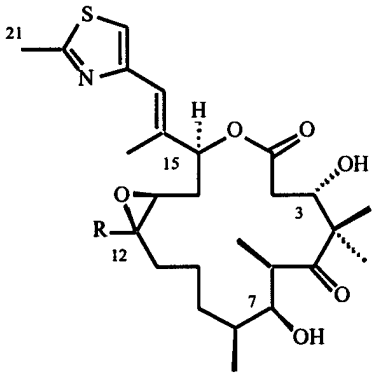
Although taxol has been approved for the treatment of breast, ovarian, and lung carcinomas, its toxicity and poor aqueous solubility, which have made formulation difficult, are continuing issues. Also, taxol is a target for P-glycoprotein, an energy dependent drug efflux pump, making it susceptible for multiple-drug-resistance (MDR). It is these undesirable characteristics of taxol, coupled with its unique mechanism of tumor inhibition that has spurred the search for novel chemotherapeutic agents with more favorable biological profiles.

Since the elucidation of the microtubule-stabilizing action of taxol in 1979 [10], nearly two decades elapsed until the discovery of new natural products which exhibit a "taxol-like" mechanism of action. The extended family of microtubule stabilizing compounds now includes the epothilones, eleutherobins, sarcodictyins, discodermolide, laulimalide, and WS9885B. Our labs have been involved in synthetic and structure-activity relationship (SAR) studies on many of these newly discovered microtubule-stabilizing agents.

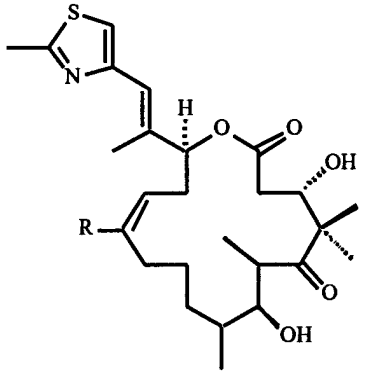
Herein, we review some of the recent developments in the synthesis and pharmacological investigation into the epothilones, eleutherobins, and related microtubule-stabilizing agents, focusing on the work done in our labs. For more comprehensive coverage on these subjects the reader is referred to other chapters and the existing literature on the subject [11].

EPOTHILONES

In 1993, the cytotoxic macrolide natural products, epothilone A (**1a**, EpoA) and B (**1b**, EpoB), along with other minor related constituents were isolated from the myxobacterium *Sorangium cellulosum* [12,13], harvested off the shores of the Zambezi River in the Republic of South Africa (Fig. (**1**)). Initial activity assessment of the epothilones demonstrated them to be potent antifungal agents [14]. In this regard, they were originally used against fungi such as *Phytophotora infestans*, which is responsible for the potato-blight disease. Needless to say, they were found to be extremely phytotoxic. It was not until 1995 that scientists at Merck discovered that the epothilones kill cells through a "taxol-like" mechanism of action [15]. However, in contrast to taxol, the epothilones inhibited the growth of MDR cell lines raising the hope that epothilone-derived anticancer drugs might eventually be useful for the treatment of drug-resistant tumors [16,17]. In addition to their promising biological profiles, the epothilones were claimed to be 30 to 50 times [13] more water soluble than taxol and are synthetically much more accessible, making



1a R = H; Epothilone A (EpoA)
1b R = Me; Epothilone B (EpoB)



2a R = H; 12,13-Desoxyepothilone A (dEpoA)
2b R = Me; 12,13-Desoxyepothilone B (dEpoB)

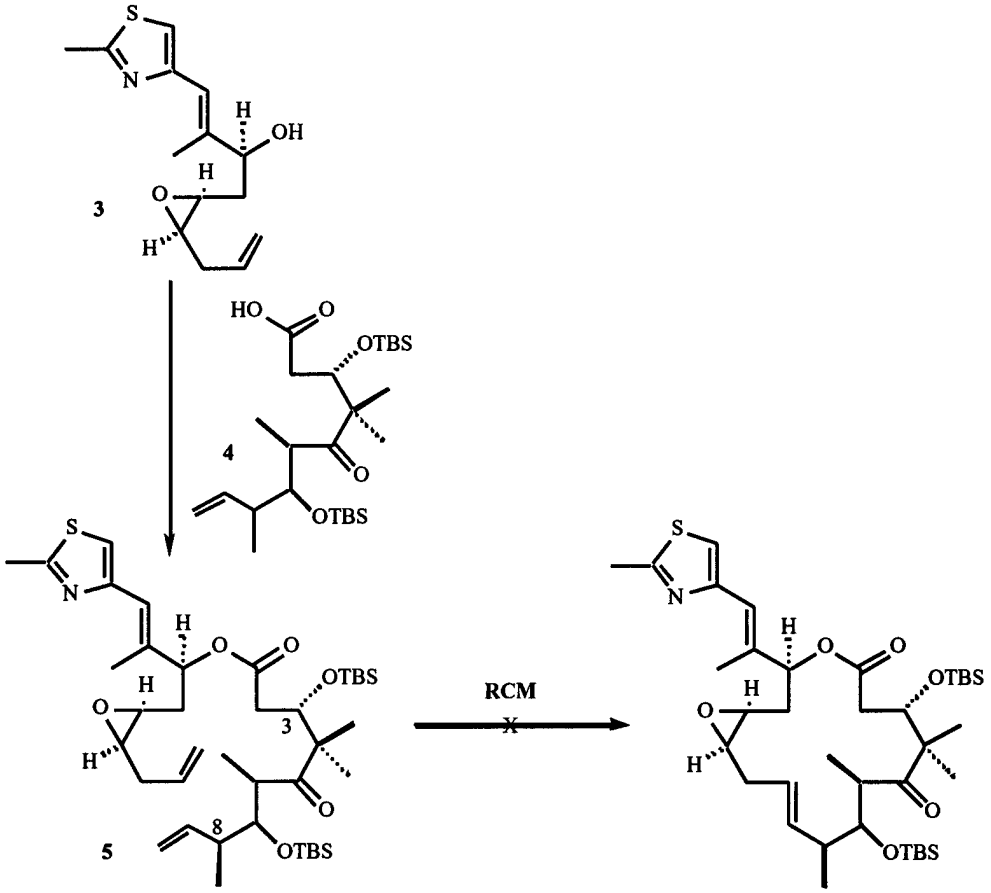
Fig. (1). The Epothilones.

analogue synthesis easier. As such, the scientific community embarked on a major effort to study and produce this valuable material.

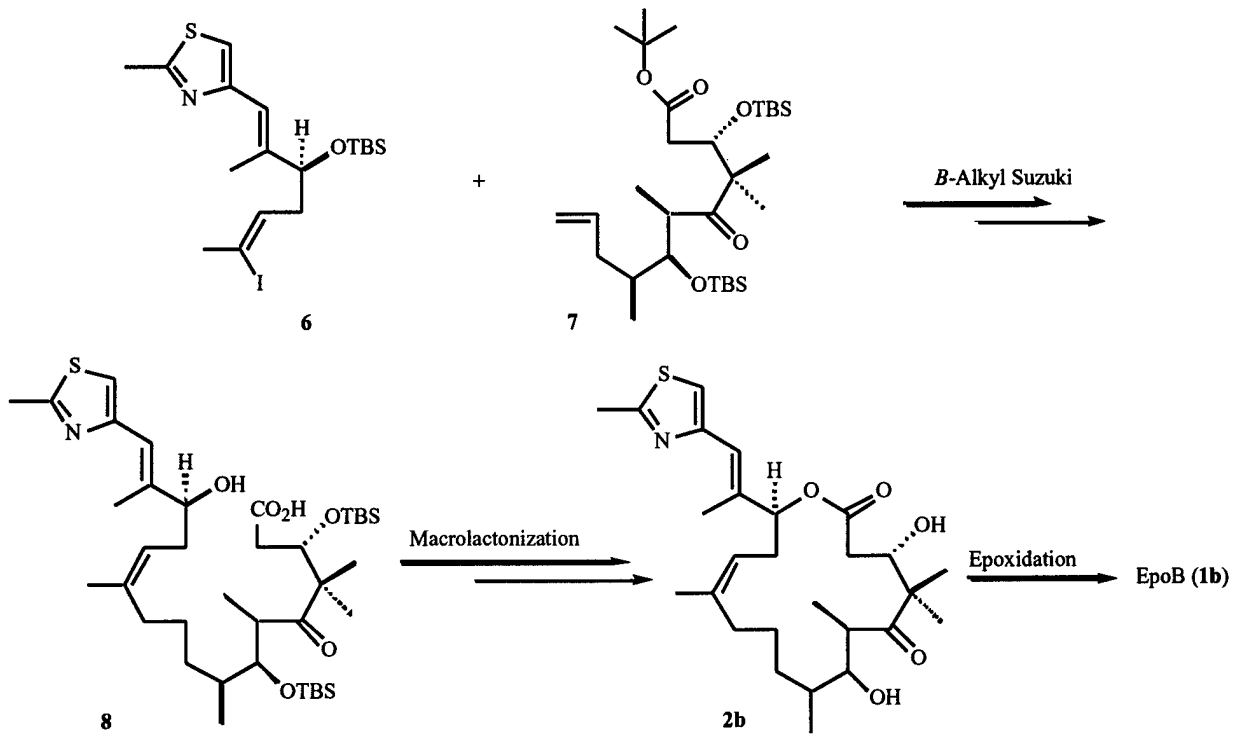
While the epothilones are available by fermentation, the more biologically active epothilone B was particularly scarce. Due to our interest in this exciting class of compounds, we embarked on securing material for further study through the efforts of total synthesis. Indeed, we accomplished the first total syntheses of epothilone A and B [18] and were soon joined by others [19]. Through *de novo* synthesis, we acquired the opportunity to explore the various regions of the molecule and provide insight into the critical functionalities necessary for biological activity, as well as functional zones amenable to chemical modifications in order to enhance its biological profile.

We explored three different methods for the closure of the 16-membered macrolide ring system; a macrolactonization approach, a macroaldolization approach, and finally a ring closing metathesis (RCM) approach. Our initial studies for the total synthesis of the epothilones was based on a ring closing metathesis reaction with the required 12,13-epoxide functionality already in place [20]. The metathesis substrate was prepared by esterification of acid **4** with epoxy alcohol **3**. Unfortunately, all attempts to effect the metathesis reaction using diene **5** failed to produce cyclized product (Scheme 1). It appears that steric congestion is detrimental for RCM reactions, and later model studies suggested that the dense functionality between C3 and C8 had undermined macrocycle formation by RCM [21].

We then turned our attention to forming the critical C11-C12 bond in the epothilone B system via a *B*-alkyl Suzuki coupling strategy (Scheme 2) [22]. Indeed, this strategy proved to be highly successful and the *B*-alkyl Suzuki coupling between vinyl-iodide **6** and β-hydroxy ester **7** was accomplished in high yield. Subsequent functional group manipulation afforded the seco acid (**8**).



Scheme 1. First attempt at ring closing metathesis reaction.



Scheme 2. Macrolactonization approach to epothilone A and B.

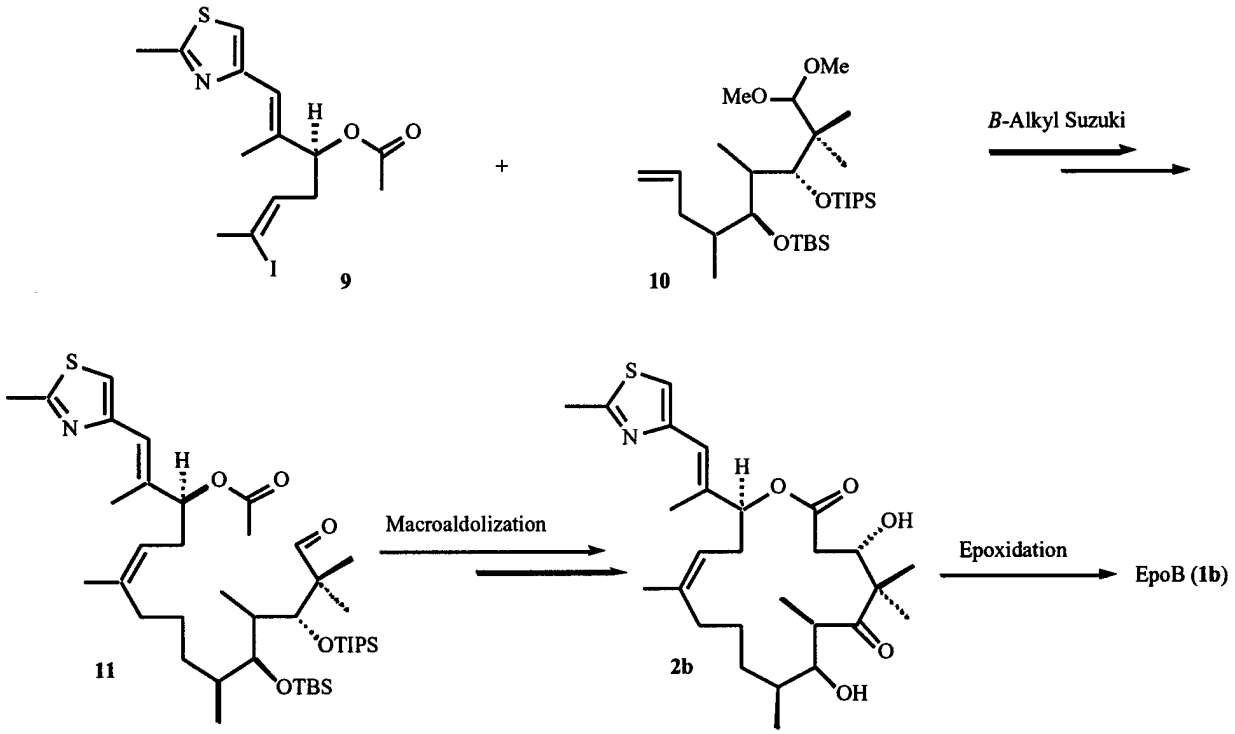
Macrolactonization was then accomplished under Yamaguchi conditions to afford the desired macrolactone in 88% yield [23]. Removal of the silyl ether protecting groups yielded 12,13-desoxyepothilone B (**2b**, dEpoB). To finish the synthesis, a regio- and stereospecific epoxidation using DMDO resulting in a > 20:1 mixture of diastereomers favoring epothilone B.

An alternate strategy for macrocyclization of the epothilone ring system, based on a macroaldolization rather than a macrolactonization, was also investigated relying on the successful *B*-alkyl Suzuki reaction between acetate **9** and dimethyl acetal **10** (Scheme 3). In the event, deprotonation of compound **11** with KHMDS in THF did indeed stereoselectively close the macrocycle, resulting in the preferential formation (6:1) of the desired (*S*)-C3 alcohol. Further functional group manipulations yielded dEpoB (**2b**) which was subsequently epoxidized to provide EpoB (**1b**).

Having successfully completed the synthesis of the epothilones we decided to reinvestigate the ring closing metathesis approach (Scheme 4). In our previous synthesis, we demonstrated that we could regio- and stereospecifically introduce the C12-13 epoxide functionality. The advent of this epoxidation allowed us to redesign the olefin metathesis substrate avoiding the use of the C9-C10 alkene whose steric hindrance had impeded the success of this approach [24]. After an intermolecular aldol reaction between acetate **12** and aldehyde **13**, we were gratified to find that olefin metathesis using diene **14** now proved successful for the ring closure, using either the ruthenium-based Grubbs catalyst [25] or the molybdenum-based Schrock catalyst [26]. Unfortunately, the ring closure, though proceeding in high yield, produced *E* : *Z* mixtures of the C12-C13 olefin which proved very difficult to separate. As such, we favored the macrolactonization approach for all subsequent analogue syntheses for the SAR studies.

BIOLOGICAL EVALUATION OF THE EPOTHILONES

Upon completion of the total synthesis, which provided epothilone A and B in sufficient quantities for detailed analysis, we set out to determine their therapeutic potential. As previously noted, our in vitro experiments showed that EpoB proved more cytotoxic than either



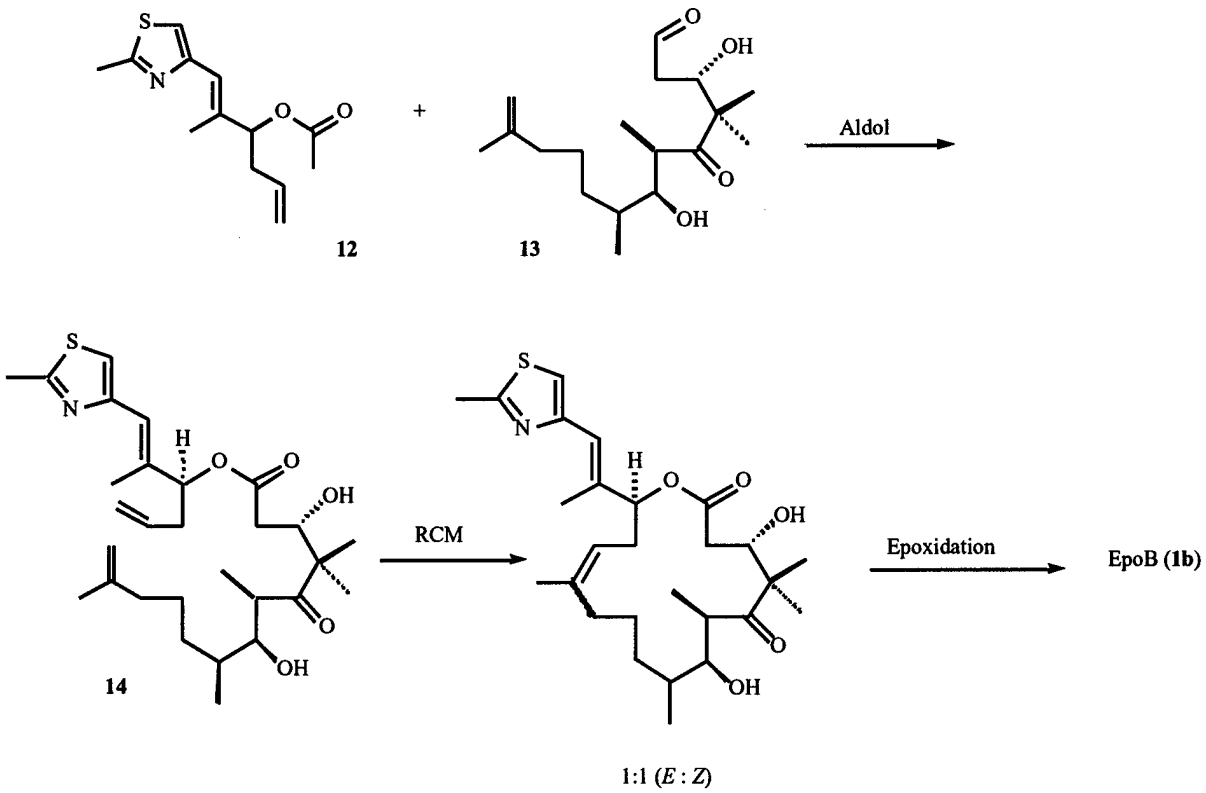
Scheme 3. Macroaldolization approach to epothilone A and B.

EpoA or paclitaxel. However, very early in vivo analyses uncovered potentially serious toxicity issues associated with EpoB. From this initial data it was determined that tolerated doses were not therapeutically adequate when compared to the clinical efficacy of paclitaxel. Undeterred by these results, we focused our efforts on developing a detailed structure-activity profile of the epothilone ring system in the hope of producing analogues with improved therapeutic potential [11a].

Having produced a practical synthesis of the epothilones with a modular design, we were able to produce a large number of other related epothilone analogues in sufficient quantities, at least for preliminary biological evaluation. The results of the SAR study obtained from our collection of analogues prompted us to divide the structure of epothilone into an acyl sector (C1-C8, zone 1), an *O*-alkyl sector (C9-C15, zone 2), and a pendent aryl sector that projects from C15 (zone 3) as depicted graphically in Fig. (**2**). These studies elicited the following summary conclusions: 1. the polypropionate sector (zone 1) constitutes a hot-spot region with great sensitivity to structural change. 2. The *O*-alkyl (zone 2) and aryl (zone 3) sectors exhibited regions of significant tolerance both in cytotoxicity and tubulin binding assays.

From the SAR analyses, we were gratified to find that one of our synthetic intermediates in the synthesis of EpoB, namely 12,13-desoxyepothilone B (**2b**, dEpoB), exhibited a favorable therapeutic profile. While dEpoB was less toxic in the in vitro cytotoxicity screens relative to EpoB, the performance of the two compounds were almost identical in the tubulin affinity assay [27]. We then proceeded to study effects of dEpoB versus EpoB in an in vivo model system. The results were astonishing. It was found that dEpoB was much less toxic than EpoB by a factor of 40. Even more encouraging was that when nude mice bearing MX-1 xenografts were treated with dEpoB (30 mg/kg, Q2Dx5, i.p.) marked tumor regression occurred and cures ensued [28].

Bolstered by the remarkable results of the initial in vivo assays with dEpoB, we continued with our assessment of its therapeutic efficacy. Since the initial evaluation, the vast superiority of dEpoB relative to EpoB and to paclitaxel has been continuously demonstrated in a variety of competitive in vivo settings. We have also



Scheme 4. Metathesis approach to the epothilones.

demonstrated the superiority of dEpoB compared with paclitaxel in multidrug-resistant tumor models [29]. Presently, dEpoB has advanced to toxicology and efficacy studies in dogs, en route to a full scale clinical evaluation.

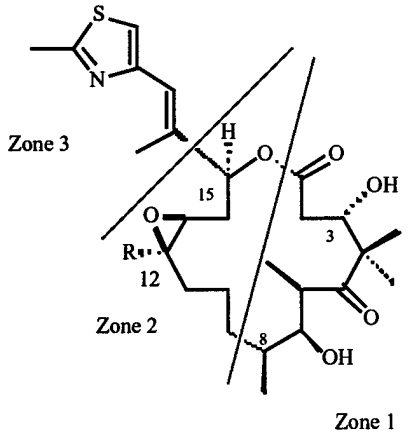


Fig. (2). Results from our SAR studies demonstrated by dividing the regions of the epothilones into zones that are susceptible to chemical modification.

Due to the unmet medical need for new chemotherapeutic agents and the promising pharmacological potential of the epothilones, a host of pharmaceutical companies have also embarked on a search for potential clinical candidates. Epothilone B itself is currently undergoing phase I clinical trials initiated by Novartis [30]. However, due to its narrow therapeutic index they continue to search for more attractive analogues. Figure (**3**) depicts some of the more effective analogues which are currently under investigation in various laboratories throughout the world. dEpoF (**15**), which contains a C21 hydroxyl functionality, is a synthetic desoxy version of the naturally occurring epothilone F. Currently, dEpoF is being advanced in our laboratories at Sloan-Kettering and has been shown to exhibit comparable efficacy to dEpoB but is more than twice as soluble in aqueous solution, which should facilitate formulation [31]. Another promising compound, **16**, in which the thiazole moiety has been locked into a defined conformation around the C17-C18 bond by a benzenoid functionality, was synthesized by chemists at Novartis Pharma AG [32]. In vitro studies have claimed **16** to be more active in vitro than dEpoB, however in vivo results have not yet been disclosed.

In the epoxide containing series, compound **17**, where the thiazole has been replaced with a 4-methyl pyridine, has also been shown to be more effective than EpoB in vitro [33]. However, it remains unclear whether such in vitro activities will be translated into in vivo efficacy since it contains the epoxide functionality which is suspected to be involved in the toxicity issues. Recently, the Bristol-Myers Squibb (BMS) company has advanced the lactam version of epothilone B (**18**) as a phase I clinical candidate [34]. The lactam is claimed to possess increased metabolic stability compared to the lactone system. In addition to the BMS report, the lactam version of the epothilones has also been independently prepared by the Schinzer lab as well as our own [35,36]. In vitro analysis, in both our and the Schinzer labs, proved the epothilone B lactam to be less potent than epothilone B [32].

Since the discovery of their microtubule-stabilizing properties, the epothilones have proven amenable to various total syntheses, and numerous analogues have been prepared. The epothilones clearly have potential advantages over taxol, both in synthetic accessibility of analogues and therapeutic profile. With the additional benefit of potent in vivo activity in taxol-resistant tumor models, the epothilones have become one of the most promising new class of compounds for the treatment of cancer. Further investigations in the synthetic, biological and medicinal arenas will demonstrate if these expectations are to be fulfilled.

ELEUTHEROBIN AND RELATED COMPOUNDS

Eleutherobin (**19**), first disclosed in the patent literature in 1995, was isolated from an *Eleutherobia* species of soft, red-colored coral found near Western Australia (Fig. (**4**)) [37]. Interest in eleutherobin was heightened by the 1997 report by Fenical regarding the mechanism of action, which involved inhibition of microtubule disassembly [38]. This microtubule-stabilizing mode of action led to immediate and intense efforts

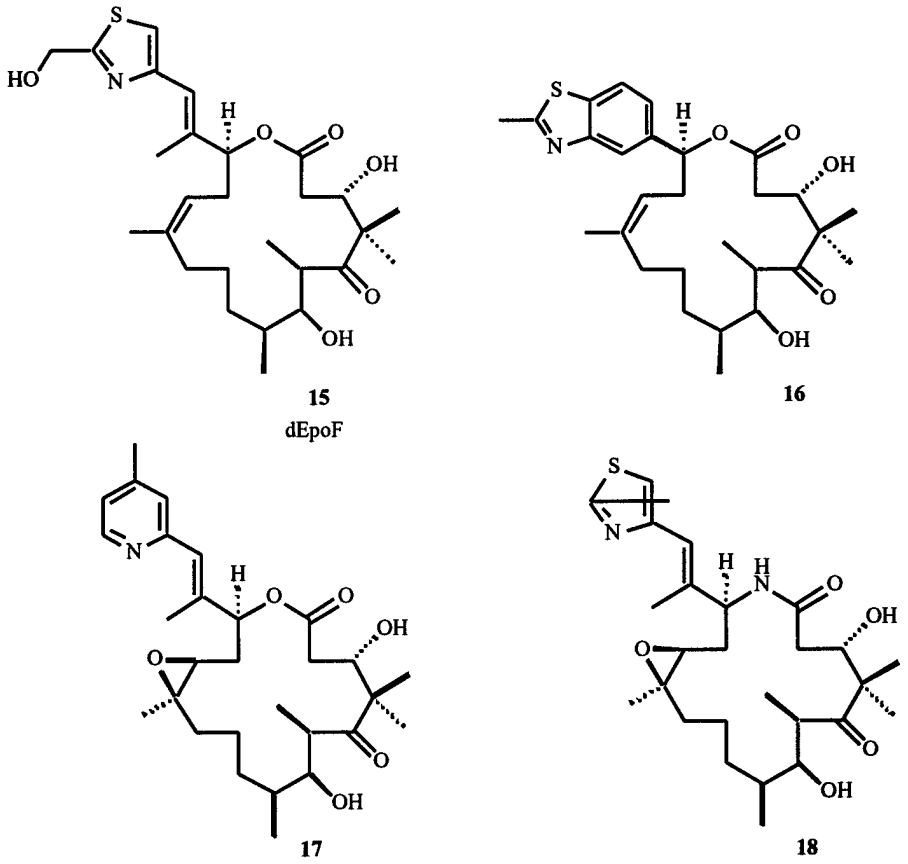


Fig. (3). Other promising epothilone analogs.

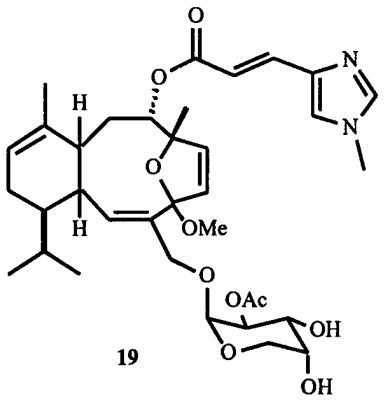


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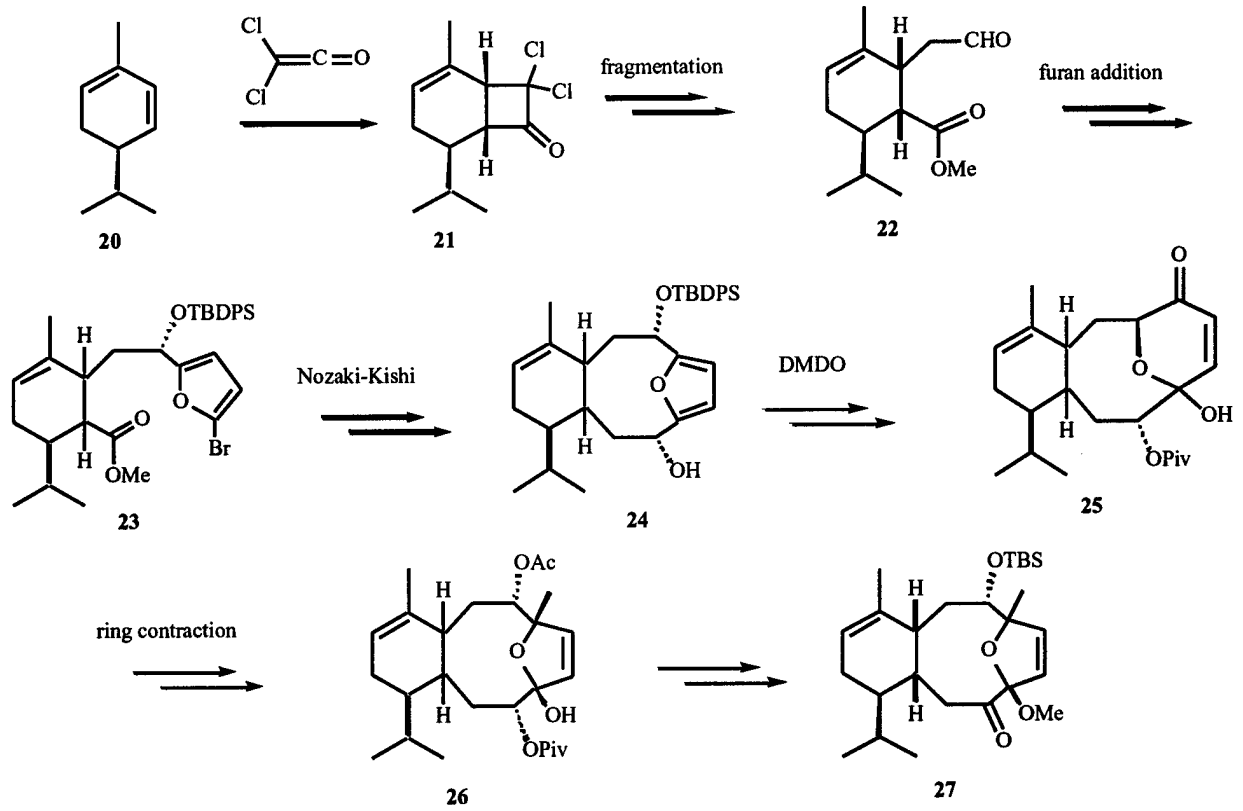
Fig. (4). Eleutherobin.

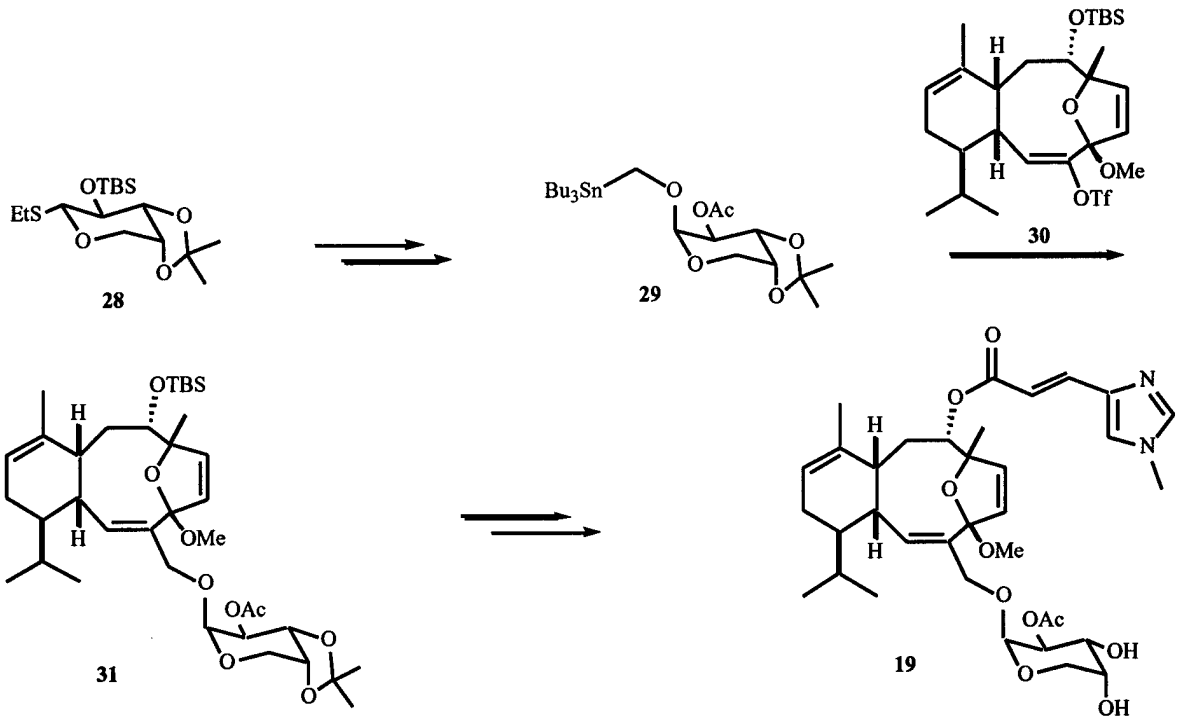
in the synthetic arena. Synthetic programs were further prompted by paucity of material available from natural sources. Hence, total synthesis would be vital for structure-activity investigations.

There have been two reported total syntheses of eleutherobin thus far [39,40], along with the disclosure of a number of approaches towards key intermediates [41]. We defined the goal of our own synthetic investigations to be that of supplying sufficient quantities of the natural product and related analogues for SAR studies [40a]. A brief account of our synthetic and subsequent biological studies is provided herein.

The synthesis of the tricyclic core structure is depicted in Scheme 5 [40a]. The synthesis began with a cycloaddition reaction involving dichloroketene and commercially available *R*-(-)-α-phellandrene (**20**). Dechlorination of **21**, followed by a Bredereck-type transformation and acid-catalyzed fragmentation afforded the intermediate **22**. The furanoid building block was appended by the addition of monolithiated dibromofuran followed by silylation to produce bromofuran **23**. Following further manipulations, a remarkable intramolecular Nozaki-Kishi coupling was successfully undertaken, affording cyclophane **24**. A ring expansion/contraction sequence completed the synthesis of the core structure. DMDO-mediated oxidation of the furanoid moiety led to the formation of the pyranose structure **25**, presumably via epoxide and diketone intermediates. Methylation and a selective acetylation of the secondary alcohol led to the generation of **26**, which was suitably differentiated for further processing. This intermediate was advanced to the ketone **27**.



Scheme 5. Synthesis of tricyclic core of eleutherobin.



Scheme 6. Completion of eleutherobin synthesis by attachment of *D*-arabinoside.

Initial experiments to install the sugar appendage with advanced arabinose donors led to consistent 1:1 mixtures of α- and β- arabinosides with various primary alcohol acceptors. This led us to conceive of and execute a new plan (Scheme 6) [40b], in which the stereochemistry of the anomeric carbon would be defined in advance, followed by the conjugation of the "oxycarba" saccharide domain to vinyl triflate **30**, obtained from ketone **27**. Conversion of the thioethyl glycoside **28** to the tri-*n*-butylstannylmethyl glycoside, followed by suitable functionalization, afforded the *D*-arabinoside **29**. A "sp^3 version" of the Stille coupling gave rise to **31**, which was elaborated to eleutherobin (**19**) in a straightforward manner, including introduction of the *N*-(1)-methyl urocanic acid side chain. We also synthesized *neo*-eleutherobin, which contained the corresponding *L*-arabinoside, and demonstrated unambiguously that the natural product had the *D*- configuration in the saccharide sector.

A structure-activity profile of synthetic eleutherobin derivatives was undertaken by us to define the structural requirements for microtubule stabilization and cross-resistance in Taxol-resistant cell lines [42]. Eleutherobin had an IC_{50} value comparable to that of taxol®, while exhibiting

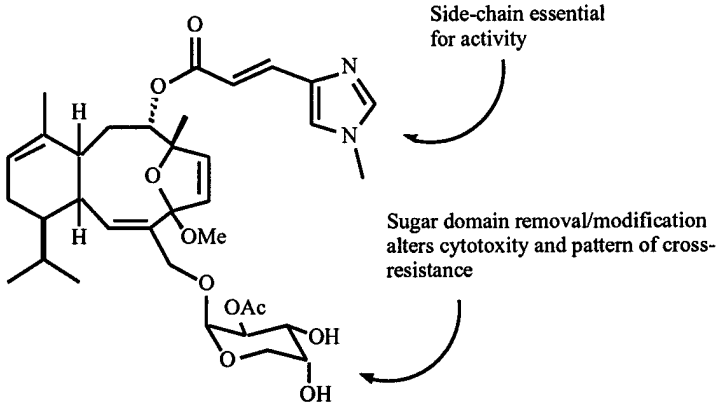


Fig. (5). SAR of eleutherobin.

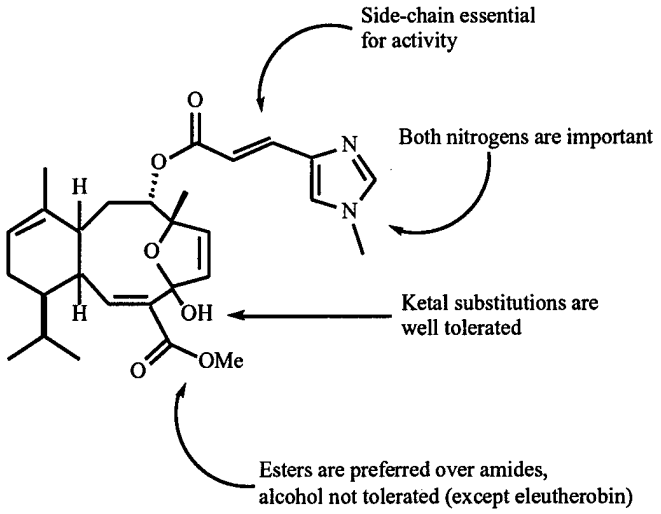


Fig. (6). SAR of sarcodictyins.

cross-resistance in *MDR1*-expressing cell lines. Thus, eleutherobin serves as a substrate for P-glycoprotein. The results of the SAR studies show that removal, or modification, of the saccharide moiety alters the cytotoxic potency of eleutherobin and its pattern of cross-resistance to the taxol-resistant cells. However, such compounds retain only a fraction of the microtubule-stabilizing activity of the native compound. The *N*-(1)-methyl urocanic acid side chain is essential for taxol-like activity. These results are summarized in Fig. (**5**).

Similar results were also obtained in a study by Nicolaou, involving the synthesis and biological evaluation of combinatorial libraries of the structurally related compound sarcodictyin [43]. A solid phase synthesis of the library was achieved, with diversity introduced in place of the side chains. The overall conclusions are depicted in Fig. (**6**), showing the importance of the urocanic acid side chain in activity.

In summary, the total synthesis of eleutherobin had been accomplished and SAR analysis performed. From the studies to date, eleutherobins synthetic complexity coupled with its MDR susceptibility have led to lowered expectations for its success as a chemotherapeutic agent.

DISCODERMOLIDE

The marine natural product discodermolide (**32,** Fig. (**7**)) was isolated from the deep-water Caribbean sponge *Discodermia dissoluta* in 1990 [44], and was initially shown to possess immunosuppressive activity [45]. It was later determined to be a potent antimitotic agent, possessing a mode of action similar to that of taxol® [46]. It arrests the cell cycle at the metaphase-anaphase transition in mitosis by microtubule stabilization. Importantly, **32** is also potent against *MDR1*-expressing cell lines, unlike taxol® and eleutherobin, but similar to the epothilones. Since only small amounts (0.009 g) could be isolated form the natural sources, total synthesis has played an important role in making material available for biological studies.

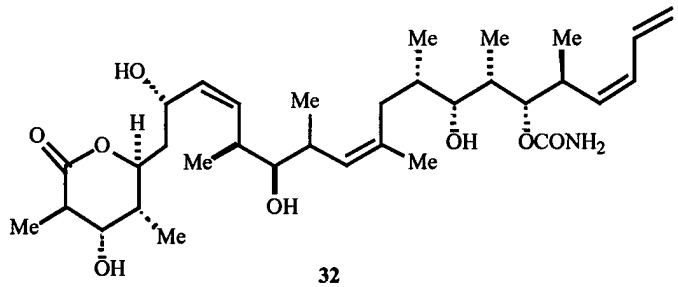


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Fig. (7). Discodermolide.

Schreiber reported the first synthesis of *ent*-**32** in 1993 [47]. This was followed by other total syntheses, including a gram-scale synthesis of discodermolide by Smith in 1999 [48]. Schreiber has also reported the synthesis of several analogues for the development of probe reagents to study the interaction between discodermolide and its receptor [49]. Recent results suggest that taxol® and discodermolide may represent a synergistic drug combination in human carcinoma cell lines, and thus could potentially constitute a promising chemotherapeutic combination [50].

LAULIMALIDE AND WS9885B

Laulimalide (**33**, Fig. (**8**)), also known as figianolide B, was isolated from various Pacific sponges [51]. It displayed potent cytotoxicity against carcinoma cell lines, with IC_{50} values in the low nanomolar range. It has also been shown to possess microtubule-stabilizing activity. In contrast to taxol®, laulimalide has been recently shown to inhibit the proliferation of SKVLB-1 cells, a P-glycoprotein expressing MDR cell line, suggesting that they are poor substrates for transport by P-glycoprotein [52]. In view of its significant clinical potential, there has been considerable interest in synthetic and structure-activity based investigations [53]. Recently, the first total synthesis of laulimalide has been reported by Ghosh [54].

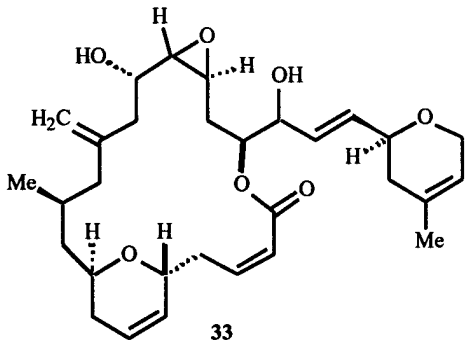
WS9885B (**34,** Figure 8) is a cytotoxic bacterial metabolite recently described by the Fujisawa Pharmaceutical Company that has also been implicated in a mechanism of action involving microtubule stabilization [55]. It has been reported to display potent taxol-like activity against several cancer cell lines.

A COMMON PHARMACOPHORE?

The ability of these structurally diverse molecules to interfere in the cell cycle by a similar mechanism has led to speculation concerning the existence a common pharmacophore that is recognized by the biological target, tubulin. The epothilones, eleutherobins, sarcodictyins and discodermolide competitively inhibit the binding of [^{3}H]paclitaxel to microtubules, indicating overlap of binding sites [56]. Independent models have been proposed in the literature regarding the possibility of a common pharmacophore [57], and it remains to be seen if these hypotheses can lead to the design of better binding and more efficacious drug analogues.

CONCLUSION

The emergence of new non-taxoid tubulin-stabilizing agents in the past decade has significant implications for the evolvement of more effective ways of treating cancer. The characterization of these compounds has led to notable progress in the synthetic and biological arenas, and the scientific community has swiftly responded to the challenge of identifying novel analogues with favorable therapeutic profiles. In our own laboratory, practical total syntheses have afforded considerable amounts of material for biological evaluations. In addition to an abundance of structure-activity related data, these investigations have led to many disclosures of synthetic accomplishments, rich with chemical dividends. With the exciting results achieved so far, the future holds promise for the development of novel anti-cancer therapies based on tubulin-stabilizing agents. Further developments are to be anticipated.



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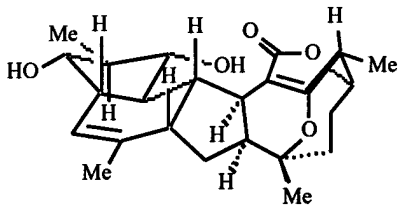

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Fig. (8). Laulimalide and WS9885B.

ACKNOWLEDGMENT

This work was supported by the National Institute of Health (Grant Numbers: AI16943, CA28824, HL25848). We thank our co-workers and collaborators, whose names appear in the references, for their invaluable contributions.

REFERENCES

[1] 'Cancer Chemotherapeutic Agents'. Ed. W. O. Foye, American Chemical Society, Washington, D.C. **1995**.

[2] Horwitz, S. B., Fant, J., Schiff, P. B. *Nature* **1979**, *277*, 665.

[3] Hyams, J. F., Loyd, C. W. *Microtubules*; Wiley-Liss: New York, 1993.

[4] Kowalski, R. J., Giannakakou, P., Hamel, E. *J. Biol. Chem.* **1997**, *272*, 2534.

[5] Timashiff, S., Andreu, J., Gorbunoff, M., Medranot, F., Prakash, V. *Cell. Pharmacol.* **1993**, *1*, S27.

[6] (a) Toso, R. J., Jordan, M. A., Farrell, K. W., Matsumoto, B., Wilson, L. *Biochemistry* **1993**, *32*, 1285. (b) Hastie, S. B. *Pharmacol. Ther.* **1991**, *51*, 377.

[7] (a) Mitchison, T., Kirschner, M. W. *Nature* **1984**, *312*, 237. (b) Kirschner, M. W., Mitchison, T. *Cell* **1986**, *45*, 329., (c) Kirschner, M. W., Mitchison, T. *Nature* **1986**, *324*, 621. (b) Sackett, D. L. *Pharmacol. Ther.* **1993**, *59*, 163.

[8] Natale, R. B. *Semin. Oncol.* **1997**, *24*, 29.

[9] Taxol is a registered trademark of Bristol-Myers Squibb.

[10] Schiff, P. B., Fant, J., Horwitz, S. B. *Nature* **1979**, *277*, 665.

[11] a)Harris, C. R., Danishefsky, S. J. *J. Org. Chem.* **1999**, *64*, 8434. (b) Nicolaou, K. C., Roschanger, F., Vourloumis, D. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2015.

[12] Gerth, K., Bedorf, N., Hofle, G., Irschik, H., Reichenbach, H. *J. Antibiot.* **1996**, *49*, 560.

[13] Hofle, G., Bedorf, N., Steinmetz, H., Schomburg, D., Gerth, K., Reichenbach, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1567.

[14] Hofle, G., Bedorf, K., Gerth, H., Reichenbach (GBF), DE-B 4138042. **1993** [*Chem. Abstr.* **1993**, *120*, 52841].

[15] Bollag, D. M., McQueney, P. A., Zhu, J., Hensens, O., Koupal, L., Liesch, J., Goetz, M., Lazarides, E., Woods, C. M. *Cancer Res.* **1995**, *55*, 2325.

[16] Kowalski, R. J., Terhaar, E., Longley, R. E., Gunasekera, S. P., Lin, C. M., Day, B. V., Hamel, E. *Mol. Biol. Cell* **1995**, *6*, 2137.

[17] Muhlradt, P. F., Sasse, F. *Cancer Res.* **1997**, *57*, 3344.

[18] (a) Meng, D., Bertinato, P., Balog, A., Su, D.-S., Kamenecka, T., Sorensen, E. J., Danishefsky, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 10073. (b) Balog, A., Meng, D., Kameneka, T., Bertinato, P., Su, D.-S., Sorensen, E. J., Danishefsky, S. J. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2801.

[19] (a) Nicolaou, K. C., He, Y., Vourloumis, D., Vallberg, H., Yang, Z. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2014 and references therein. For recent chemical syntheses of epothilone B. see: (b) Sawada, D., Kanai, M., Shibasaki, M. *J. Am. Chem. Soc.* **2000**, *122*, 10521. (c) Mulzer, J., Mantoulidis, A., Ohler, E. *J. Org. Chem.* **2000**, *65*, 7456. (d) White, J. D., Carter, R. G., Sundermann, K. F. *J. Org. Chem.* **1999**, *64*, 684. (e) May, S. A., Grieco, P. A. *Chem. Comm.* **1998**, 1597.

[20] Meng, D., Bertinato, P., Balog, A., Su, D.-S., Kamenecka, T., Sorensen, E. J., Danishefsky, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 10073

[21] For a review of ring-closing olefin metathesis see: (a) Grubbs, R. H., Miller, S. J., Fu, G. C. *Acc. Chem. Res.* **1995**, *28*, 446. (b) Schmalz, H.-G. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1833.

[22] (a) Miyaura, N,; Ishiyama, T., Sasaki, H., Ishi-Kawa, M., Satoh, M., Suzuki, A. *J. Am. Chem. Soc.* **1989**, *111*, 314. (b) For an excellent review see: Miyaura, N., Suzuki, A. *Chem Rev.* **1995**, *95*, 2457.

[23] (a) Yamaguchi, M., Inanaga, J., Hirata, K., Saeki, H., Katsuki, T. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989. (b) Mulzer, J., Mareski, P. A., Buschmann, J., Luger, P. *Synthesis* **1992**, 215.

[24] Meng, D., Su, D.-S., Balog, A., Bertinato, P., Sorensen, E. J., Danishefsky, S. J., Zheng, Y.-H., Chou, T.-C., He, L., Horwitz, S. B. *J. Am. Chem. Soc.* **1997**, *119*, 2733.

[25] Schwab, P., France, M. B., Ziller, J. W., Grubbs, R. H. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2039.

[26] Schrock, R. R., Murdzek, J. S., Bazan, G. C., Robbins, J., DiMare, M., O'Regan, M. *J. Am. Chem. Soc.* **1990**, *112*, 3875.

[27] Chou, T.-C., Zhang, X.-G., Balog, A., Su, D.-S., Meng, D., Savin, K., Bertino, J. R., Danishefsky, S. J. *Proc. Natl. Acad. Sci.* **1998**, *95*, 9642.

[28] MX-1 is a well characterized human mammary adenocarcinoma that has been used for many years as part of the National Cancer Institute's screening panel of experimental tumors.

[29] Chou, T.-C., Zhang, X. G., Harris, C. R., Kuduk, S. D., Balog, A., Savin, K., Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15798.

[30] Calvert, P. M., O'Neill, V., Azzabi, A., Hughes, A., Plummer, R., Twelves, C., Robinson, A., Machan, M. A., Dimitrijevic, S., Moss, D., Rothermel, J., Cohen, P., Chen, T., Man, A., Calvert, A. H. *Clin. Cancer Res.* **2000**, *6*, 4581S.

[31] Lee, C. B; Chou, T. C; Zhang, X. G; Wang, Z. G; Kuduk, S. D., Chappel, M. D., Stachel, S. J., Danishefsky, S. J. *J. Org. Chem.* **2000**, *65*, 6525.

[32] Altmann, K.-H., Bold, G., Caravatti, G., End, N., Florsheimer, A., Guagnano, V., O'Reilly, T., Wartmann, M. *Chemia* **2000**, *54*, 612.

[33] Nicolaou, K. C., Scarpelli, R., Bollbuck, B., Werschkun, B., Pereira, M. M. A., Wartmann, M., Altmann, K. –H., Zaharevitz, D., Gussio, R., Giannakakou, P. *Chemistry & Biology* **2000**, *7*, 593.

[34] (a) Vite, G. D., Borzilleri, R. M., Kim, S.-H., Johnson, J. A., Patent WO99/102514, **1999**. (b) Borzilleri, R. M., Zheng, X., Schmidt, R. J., Johnson, J., Kim, S.-H., DiMarco, J. D., Fairchild, C. R., Gougoutas, J. Z., Lee, F. Y. F., Long, B. H., Vite, G. D. *J. Am. Chem. Soc.* **2000**, *122*, 8890.

[35] Schinzer, D., Altmann, K. H., Stuhlmann, F., Bauer, A., Wartmann, M. *Chembiochem* **2000**, *1*, 67.

[36] Stachel, S. J., Chappell, M. D., Lee, C. B., Danishefsky, S. J., Chou, T.-C., He, L., Horwitz, S. B. *Org. Lett.* **2000**, *11*, 1637.

[37] Fenical, W.-H., Jensen, P. R., Lindel, T. U.S. patent 5,473,057, **1995**.

[38] (a) Lindel, T., Jensen, P. R., Fenical, W., Long, B. H., Casazza, A. M., Carboni, J., Fairchild, C. R. *J. Am. Chem. Soc.* **1997**, *119*, 8744. (b) Long, B. H., Carboni, J. M., Wasserman, A. J., Cornell, L. A., Casazza, A. M., Jensen, P. R., Lindel, T., Fenical, W.-H., Fairchild, C. R. *Cancer Res.* **1998**, *58*, 1111.

[39] (a) Nicolaou, K. C., van Delft, F. L., Ohshima, T., Vourloumis, D., Xu, J. Y., Hosokawa, S., Pfefferkorn, J., Kim, S., Li, T. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2520. (b) Nicolaou, K. C., Ohshima, T., Hosokawa, S., van Delft, F. L., Vourloumis, D., Xu, J. Y., Pfefferkorn, J., Kim, S. *J. Am. Chem. Soc.* **1998**, *120*, 8674.

[40] (a) Chen, X.-T., Gutteridge, C. E., Bhattacharya, S. K., Zhou, B., Pettus, T. R. R., Haskall, T., Danishefsky, S. J. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 185. (b) Chen, X.-T., Zhou, B., Bhattacharya, S. K., Gutteridge, C. E., Pettus, T. R. R., Danishefsky, S. J. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 789. (c) Chen, X.-T., Bhattacharya, S. K., Zhou, B., Gutteridge, C. E., Pettus, T. R. R., Danishefsky, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 6563.

[41] (a) Ceccarelli, S., Piarulli, U., Gennari, C. *Tetrahedron Lett.* **1999**, *40*, 153. (b) Baron, A., Caprio, V., Mann, J. *Tetrahedron Lett.* **1999**, *40*, 9321. (c) Carter, R., Hodgetts, K., Mckenna, J., Magnus, P., Wren, S. *Tetrahedron* **2000**, *56*, 4367.

[42] McDaid, H. M., Bhattacharya, S. K., Chen, X.-T., He, L., Shen, H.-J., Gutteridge, C. E., Horwitz, S. B., Danishefsky, S. J. *Cancer Chemother. Pharmacol.* **1999**, *44*, 131.

[43] Nicolaou, K. C., Winssinger, D., Vourloumis, D., Ohshima, T., Kim, S., Pfefferkorn, J., Xu, J.-Y., Li., T. *J. Am. Chem. Soc.* **1998**, *120*, 10814.

[44] Gunasekara, S. P., Gunasekara, M., Longley, R. E. *J. Org. Chem.* **1990**, *55*, 4912 (correction: *J. Org. Chem.* **1991**, *56*, 1346).

[45] Longley, R. E., Caddigan, D., Harmody, D., Gunasekara, M., Gunasekara, S. P. *Transplatation* **1991**, *52*, 650.

[46] (a) Terhaar, E., Kowalski, R. J., Hamel, E., Lin, C. M., Longley, R. E., Gunasekara, S. P., Rosenkrantz, H. S., Day, B. W. *Biochemistry* **1996**, *35*, 243. (b) Hung, D. T., Chen, J., Schreiber , S. L. *Chem. Biol.* **1996**, *3*, 287.

[47] Nerenberg, J. B., Hung, D. T., Somers, P. K., Schreiber, S. L. *J. Am. Chem. Soc.* **1993**, *115*, 12621.

[48] (a) Smith, A. B., Qui, Y., Jones, D. R., Kobayashi, K. *J. Am. Chem. Soc.* **1995**, *117*, 12011. (b) Harried, S. S., Yang, G., Strawn, M. A., Myles, D. C. *J. Org. Chem.* **1997**, *62*, 6908. (c) Marshall, J. A., Johns, B. A. *J. Org. Chem.* **1998**, *63*, 7885. (d) Smith, A. B., Kaufman, M. D., Beauchamp, T. J., LaMarche, M. J., Arimoto, H. *Org. Lett.* **1999**, *1*, 1823.

[49] Hung, D. T., Nerenberg, J. B., Schreiber, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 11054.

[50] Martello, L. A., McDaid, H. M., Regl, D. L., Yang, C. P., Meng, D., Pettus, T. R. R., Kaufman, M. D., Arimoto, H., Danishefsky, S. J., Smith, A. B., Horwitz, S. B. *Clin. Cancer Res.* **2000**, *6*, 1978.

[51] (a) Quinoa, E., Kakou, Y., Crews, P. *J. Org. Chem.* **1988**, *53*, 3642. (b) Corley, D. G., Herb, R., Moore, R. E., Scheuer, P. J., Paul, V. J. *J. Org. Chem.* **1988**, *53*, 3644. (c) Jefford, C. W., Bernardinelli, G., Tanaka, J.-I., Higa, T. *Tetrahedron Lett.* **1996**, *37*,

159. (d) Tanaka, J.-I., Higa, T., Bernardinelli, G., Jefford, C. W. *Chem. Lett.* **1996**, 255.

[52] Moorberry, S. L., Tien, G., Hernandez, A. H., Plubrukarn, A., Davidson, B. S. *Cancer Res.* **1999**, *59*, 653.

[53] (a) Mulzer, J., Hanbauer, M., *Tetrahedron Lett.* **2000**, *41*, 33. (b) Ghosh, A. K., Wang, Y. *Tetrahedron Lett.* **2000**, *41*, 2319. (c) Ghosh, A. K., Wang, Y. *Tetrahedron Lett.* **2000**, *41*, 4705.

[54] Ghosh, A. K., Wang, Y. *J. Am. Chem. Soc.* **2000**, *122*, 11027.

[55] Muramatsu, H., Miyauchi, M., Sato, B., Yoshimura, S. 40th Symposium on the Chemistry of Natural Products, Fukuoka, Japan, **1998**, paper 83, p. 487.

[56] (a) Kowalski, R. J., Giannakakou, P., Hamel, E. *J. Biol. Chem.* **1997**, *272*, 2534. (b) Kowalski, R. J., Ter Haar, E., Longley, R. E., Gunasekara, S. P., Lin, C. M., Day, B. W., Hamel, E. *Mol. Biol. Cell.* **1995**, *6*, 368a.

[57] (a) Ojima, I., Chakravarty, S., Inoue, T., Lin, S., He, L., Horwitz, S. B., Kuduk, S. D., Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4256. (b) Giannakakou, P., Gussion, R., Nogales, E., Downing, K. H., Zaharevitz, D., Bolbuck, B., Poy, G., Sackett, D., Nicolaou, K. C., Fojo, T. . *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 2904. (c) He, L., Jagtap, P. G., Kingston, D. G., Shen, H. J., Orr, G. A., Horwitz, S. B. *Biochemistry* **2000**, *39*, 3972.

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Published on Web 06/22/2002

Highly Concise Routes to Epothilones: The Total Synthesis and Evaluation of Epothilone 490

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Received March 19, 2002

Abstract: A concise modular laboratory construction of the epothilone class of promising antitumor agents has been accomplished. For the first time in the epothilone area, the new synthesis exploits the power of ring-closing olefin metathesis (RCM) in a stereospecific way. Previous attempts at applying RCM to epothilone syntheses have been repeatedly plagued by complete lack of stereocontrol in the generation of the desired 12,13-olefin geometry in the products. The isolation of epothilone 490 (**3**) prompted us to reevaluate the utility of the RCM procedure for fashioning the 10,11-olefin, with the *Z*-12,13-olefin geometry already in place. Olefin metathesis of the triene substrate **12** afforded the product diene macrolide in stereoselective fashion. For purposes of greater synthetic convergency, the C3-(*S*)-alcohol was fashioned late in the synthesis, using chiral titanium-mediated aldol conditions with the entire *O*-alkyl fragment as a C15 acetate as the enolate component. Examination of the effects of protecting groups on the RCM process showed that deprotection of the C7 alcohol has a beneficial effect on the reaction yield. Performing the RCM as the last synthetic step in the sequence afforded a 64% yield of only the desired *E*-olefin. Selective diimide reduction of the new 10,11-olefin yielded 12,13-desoxyepothilone B, our current clinical candidate, demonstrating the utility of this new RCM−reduction protocol in efficiently generating the epothilone framework. Furthermore, the new olefin was selectively funtionalized to demonstrate the advantage conferred by this route for the construction of new analogues for SAR studies, in cytoxicity and microtubule affinity screens. Also described is the surprisingly poor in vivo performance of epothilone 490 in xenografts in the light of very promising in vitro data. This disappointing outcome was traced to unfavorable pharmacokinetic features of the drug in murine plasma. By the pharmacokinetic criteria, the prognosis for the effectiveness of **3** in humans is, in principle, much more promising.

Introduction

Microtubules are polymeric structures that are an integral part of all eukaryotic cells.[1] During mitosis, the dynamics of microtubule polymerization and depolymerization are finely controlled, and any variation in the rate of polymerization can profoundly effect cellular replication. Effected cells are unable to pass through a checkpoint in the cell cycle and enter into programmed cell death. There are two major classes of chemotherapeutic agents that induce mitotic arrest by disrupting microtubule dynamics, those that depolymerize tubulin and those that stabilize tubulin polymers. The most well-known tubulin-stabilizing agent is Taxol (paclitaxel), which is currently a front-line anticancer agent.[2] The current clinical success of Taxol, and the related taxane Taxotere (docetaxel), in combating a variety of human carcinomas has indeed been suggestive of the potential efficacy of other tubulin-stabilizers in cancer therapy.

However, Taxol is less than an ideal drug. Its shortcomings include multidrug resistance (MDR) susceptibility and lack of aqueous solubility. The latter condition compels recourse to formulation vehicles such as cremophores, which have their own associated toxicities.[3] Given the well-established usefulness of

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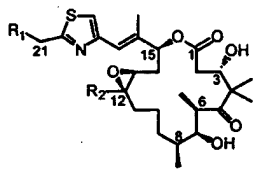
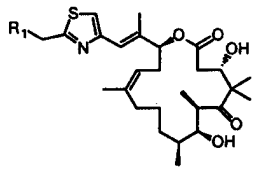
[§] Department of Molecular Pharmacology, Albert Einstein College of Medicine.
[⊥] Department of Chemistry, Columbia University.

(1) Avila, J.; Nido, J. D. In *Cytoskeleton*; Hesketh, J. E., Pryme, I. F., Eds.; JAI, Greenwich, CT, 1995; Vol. 1, p 47.
(2) Nicolaou, K. C.; Dai, W.-M.; Guy, R. K. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 15.
(3) (a) Rowinsky, E. K.; Eisenhauer, E. A.; Chaudhry, V.; Arbuck, S. G.; Donehawer, R. C. *Semin. Oncol.* **1993**, *20*, 1. (b) Fletcher, B. S.; Kujubadu, D. A.; Perrin, D. M.; Herschman, H. R. *J. Biol. Chem.* **1992**, *267*, 4338. (c) Tsuji, M.; Dubois, R. N. *Cell* **1995**, *3*, 493. (d) Essayan, D. M.; Kagey-Sobotka, A.; Colarusso, P. J.; Lichtenstein, L. M.; Ozols, R. F.; King, E. D. *J. Allergy Clin. Immunol. I* **1996**, *97*, 42. (e) Giannakakou, P.; Sackett, D. L.; Kang, Y.-K.; Zhan, Z.; Buters, J. T.; Fojo, T.; Poruchynsky, M. S. *J. Biol. Chem.* **1997**, *272*, 17118 and references therein.

10.1021/ja0262333 CCC: $22.00 © 2002 American Chemical Society

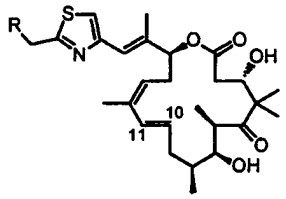


R_1, R_2 = H, epothilone A
R_1 = H, R_2 = CH_3, epothilone B
R_1 = OH, R_2 = CH_3, epothilone F



1 R_1 = H, 12,13-desoxyepothilone B
2 R_1 = OH, 12,13-desoxyepothilone F

Figure 1. Structures of epothilones.



3, R = H, epothilone 490
4, R = OH, 10,11-dehydrodesoxyepothilone F

Figure 2. Structures of 10,11-dehydroepothilones.

Taxol against a variety of oncological indications, it is not surprising that there has been a continuing interest in agents which share the mechanism of action of Taxol while offering potential therapeutic advantages. One target of opportunity would be the discovery of a drug, operating in the mechanistic framework of Taxol, which exhibits a much greater robustness toward disablement via the onset of multidrug resistance.

Such considerations were, doubtless, involved in research which led to the discovery of the family of novel macrolides, the epothilones. These compounds were isolated from the cellulose-degrading myxobacterium, *Sorangium cellulosum*, harvested off the shores of the Zambezi river in South Africa in the late 1980s (Figure 1). Initial disclosure of antifungal activity in 1993[4] was followed by reports of "Taxol-like" microtubule stabilizing-capability induced cytotoxicity in 1995.[5] Our total syntheses of the first discovered epothilones, A and B (Figure 1),[6] provided material for corroborating in vitro studies as well as for the first published in vivo evaluations.[7] Modifications of our early total syntheses also provided congeners for SAR mapping.[8] Following concerns about serious toxicities noted in our in vivo studies of fully synthetic epothilone B, we came to examine 12,13-desoxyepothilone B (dEpoB, **1**, Figure 1) in which the epoxide, a potentially serious source of indiscriminate cytotoxicity, is deleted. On the basis of its extensive and highly encouraging package of preclinical data in mice and dogs, **1** has emerged as a rather promising candidate for more advanced oncological assessments.[9] Indeed, dEpoB is presently being evaluated in human clinical trials. The compound is currently produced either through genetically engineered bacterial fermentation[10] or via chemical total synthesis. The latter method has also produced ample quantities of **1** of suitable purity and toxicology characteristics to be acceptable for extensive human clinical studies. The 21-hydroxy derivative, epothilone F, has also been of interest because of added aqueous solubility, a major problem in paclitaxel administration. We have synthesized and shown that 12,13-desoxyepothilone F (**2**) is equally effective in treatment of murine tumor xenografts as dEpoB, and superior to other clinically advanced derivatives.[9,11]

The progress of a pharmaceutically relevant investigational program in our laboratory setting is critically dependent upon facile access to significant quantities of purified material for preclinical and clinical evaluations. Our initial academic level efforts in the epothilone arena were rewarding and intellectually enriching, especially with regard to the application of LACDAC reactions developed in the 1980s in this laboratory for the stereoselective construction of carbon frameworks with programmable relative and absolute stereochemistry.[12] However, faced with pressing needs of substantial quantities of various epothilones for biological evaluation and with not having access to fermentation-derived material, we have been revisiting the matter of total synthesis on a continuing basis. Our studies are directed in the first instance to new strategy-level solutions. Significant thematic departures have indeed been developed and applied to the epothilones and have provided efficient access to multigram quantities of purely synthetic material to enable drug-discovery and evaluation campaigns.[13]

Several considerations drove the research described below. First, we continue to seek syntheses which could be suitable for eventual large-scale manufacture of desoxyepothilone B. At the same time, we hoped that the new synthesis would provide access to explore novel epothilones, in particular, the recently isolated macrolide, epothilone 490 (**3**, Figure 2).[14] This com-

(4) Höfle, G.; Bedorf, N.; Gerth, K.; Reichenbach, H. (GBF). DE-B 4138042; *Chem. Abstr.* **1993**, *120*, 52841.
(5) (a) Bollag, D. M.; McQueney, P. A.; Zhu, J.; Hensens. O.; Koupal, L.; Leisch, J.; Goetz, M.; Lazarides. E.; Woods, C. M. *Cancer Res.* **1995**, *55*, 2325. (b) Kowalski, R. J.; Terhaar. E.; Longley, R. E.; Gunasekera, S. P.; Lin, C. M.; Day, B. V.; Hamel, E. *Mol. Biol. Cell* **1995**, *6*, 2137.
(6) (a) Balog, A.; Meng, D. F.; Kamenecka, T.; Bertinato, P.; Su, D.-S.; Sorensen, E. J.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2801. (b) Su, D.-S.; Meng, D. F.; Bertinato, P.; Balog, A., Sorensen; E. J.; Danishefsky, S. J.; Zheng, Y. H.; Chou, T. C.; He, L. F.; Horwitz, S. B. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 757. (c) Meng, D. F.; Bertinato, P.; Balog, A.; Su, D.-S.; Kamenecka, T.; Sorensen, E. J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 10073. For initial reports of other epothilone syntheses, see: (d) Yang, Z.; He, Y.; Vourloumis, D.; Vallberg, H.; Nicolaou, K. C. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 166. (e) Nicolaou, K. C.; Sarabia, F.; Ninkovic, S.; Yang, Z. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 525. (f) Nicolaou, K. C.; Winssinger, N.; Pastor, J.; Nincovic, S.; Sarabia, F.; He, Y.; Vourloumis, D.; Yang, Z.; Li, T.; Giannakakou, P.; Hamel, E. *Nature* **1997**, *387*, 268. (g) Schinzer, D.; Limberg, A.; Bauer, A.; Bohm, O. M.; Cordes, M. *Angew. Chem.. Int. Ed. Engl.* **1997**, *36*, 523. (h) May, S. A.; Greico, P. A. *Chem. Commun.* **1998**, 1597. (i) Sawada, D.; Shibasaki, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 209. (j) Martin, H. J.; Drescher, M.; Mulzer, J. *Angew. Chem., Int. Ed.* **2000**, *39*, 581. (k) White, J. D.; Carter, R. G.; Sundermann, K. F. *J. Org. Chem.* **1999**, *64*, 684. (l) Zhu, B.; Panek, J. S. *Org. Lett.* **2000**, *2*, 2575, (m) Bode, J. W.; Carreira, E. M. *J. Am. Chem. Soc.* **2001**, *123*, 3611. (n) Fürstner, A.; Mathes, C.; Grela, K. *Chem. Commun.* **2001**, *12*, 1057. (o) Taylor, R. E.; Chen, Y. *Org. Lett.* **2001**, *3*, 2221. (p) Valluri, M.; Hindupur, R. M.; Bijoy, P.; Labadie, G.; Jung, J. C.; Avery, M. A. *Org. Lett.* **2001**, *3*, 3607. (q) Ermolenko, M. S.; Potier, P. *Tetrahedron Lett.* **2002**, *43*, 2895.
(7) (a) Chou. T. C.; Zhang, X. G.; Harris, C. R.; Kuduk, S. D.; Balog, A.; Savin, K. A.; Bertino, J. R.; Danishefky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15798. (b) Chou, T. C.; Zhang, X. G.; Balog, A.; Su, D.; Meng, D. F.; Savin, K.; Bertino, J. R.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9642.
(8) Harris, C. R.; Danishefsky, S. J. *J. Org. Chem.* **1999**, *64*, 8434 and references therein.
(9) Chou, T. C.; O'Connor, O. A.; Tong, W. P.; Guan, Y.; Zhang, Z.-G.; Stachel, S. J.; Lee, C.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8113.
(10) Tang, L.; Shah, S.; Chung, L.; Carney, J.; Katz, L.; Khosla, C.; Julien, B. *Science* **2000**, *287*, 640.
(11) (a) Lee, C. B.; Chou, T.-C.; Zhang, X. G.; Wang, Z. G.; Kuduk, S. D.; Chappell, M. D.; Stachel, S. J.; Danishefsky, S. J. *J. Org. Chem.* **2000**, *65*, 6525. (b) Lee, C. B.; Wu, Z.; Zhang, F.; Chappell, M. D.; Stachel, S. J.; Chou, T. C.; Guan, Y.; Danishefksy, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 5249.
(12) (a) Danishefsky, S. J. *Aldrichimica Acta* **1986**, *19*, 59. (b) Danishefsky, S. J. *Chemtracts: Org. Chem.* **1989**, *2*, 273.
(13) Harris, C. R.; Kuduk, S. D.; Balog, A.; Savin, K.; Glunz, P.; Danishefksy, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 7050.
(14) Kosan Biosciences Inc., Hayward, CA, private communication. The related 12-desmethy-10,11-dehydro compound, in the epo A series, has also been isolated recently; see: Hardt, I. H.; Steinmetz, H.; Gerth, K.; Sasse, F.; Reichenbach, H.; Hofle, G. *J. Nat. Prod.* **2001**, *64*, 847.

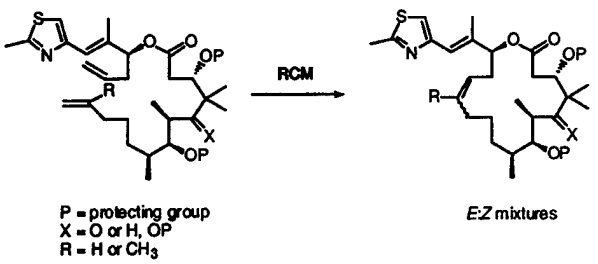


Figure 3. Previous attempts at synthesis of epothilones employing RCM for macrolide contruction, affording *E*:*Z* mixtures of the C12–C13 olefin.

pound, which contains a 10,11-*E*-olefin conjugated to the usual 12,13-unsaturation of dEpoB, showed highly favorable in vitro cytotoxicity in preliminary screens. The prospects for a quality synthesis of epothilone 490 from dEpoB were not inviting.

From a purely synthetic perspective, the presence of the C10–C13 diene unit was suggestive of potential forays into new ways of macrocyclization toward the synthesis of epothilones. Olefin metathesis certainly stands out as a premier methodological advance in carbon–carbon bond formation reactions of recent vintage.[15] Along with the dramatic advances in the field of olefin metathesis, the demonstration of the applicability of ring-closing olefin metathesis (RCM) in the assembly of a complex macrolide by Hoveyda and co-workers in the context of Sch 38516 has opened up new avenues for large-ring construction.[16] Following these elegant studies, significant efforts had been directed to using olefin metathesis for establishing the 12,13-double bond of the epothilones from the earliest days of the problem. Unfortunately, RCM directed to the 12,13-linkage has invariably led to a stereorandom mixture of olefin isomers, separable only with the greatest of difficulty (Figure 3).[6c,f–h]

It was from this background, we considered the possibility of using RCM to construct a 10,11-double bond *in a substrate which already contains the putative 12,13-unsaturation*. In proposing this solution to the problem, we were not unmindful of a recent success enjoyed in our laboratory in the total synthesis of radicicol using RCM to fashion a lactonic diene,[17] along with other recent examples of macrocyclization approaches involving diene–ene RCM.[18] Successful olefin metathesis would produce a 10,11;12,13-diene of the type found in epothilone 490. We also hoped that position-selective hydrogenation of the disubstituted 10,11-olefin would provide access to the 12,13-desoxyepothilones themselves. As seen below, this new strategy for synthesizing epothilones has been realized. When combined with a new and highly convergent way to join key pre-epothilone fragments, a highly modular synthesis from readily available modules has been realized. The prospects for a synthesis of dEpoB which would be practical at the plant level is much enhanced.

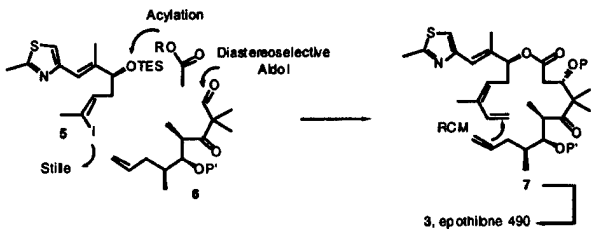


Figure 4. Synthetic plan for epothilone 490.

Specifically, at the level of chemical synthesis, we report (i) the first total synthesis of the potentially important epothilone 490 (**3**) and the related 21-hydroxy derivative (**4**) via the nonprecedented application of highly stereoselective RCM to this series of drug prospects, (ii) application to a particularly straightforward total synthesis of the highly promising 12,13-desoxyepothilone B, and (iii) exploitation of the new olefinic functionality of epothilone 490 to reach novel epothilones. Moreover, we report in vitro as well as the first in vivo assessment of **3**. Pharmacokinetic studies of fully synthetic epothilone 490 help place these findings in xenografts in perspective.

Results and Discussion

The synthetic plan envisaged a construction of a "seco" acyclic triene (**7**, Figure 4) positioned for diene–ene RCM for macrolide formation. Fortunately, we could draw upon previously disclosed and highly accessible building blocks to pursue a new vision of the epothilone synthesis problem. These are vinyl iodide **5**,[19] and aldehyde **6**.[11b,20] Inspection of the relationship of these two building blocks to goal structure **3** obliges one to deal with two issues. In terms of gross carbon count, there is a need to insert carbons 1 and 2 of the eventual epothilone into the regime, formally as a carboxymethyl spacer function. With C1 and C2 inserted, the carbon network of the two-component array **5** and **6**, while of the appropriate length, is not well-structured to deliver the 10,11-unsaturation of epothilone 490. An intriguing possibility was that of introducing two more carbon based centers to the ensemble. We envisioned that RCM would deliver the conjugated diene linkage of 490 while disposing of the extraneous carbons (vide infra).

The "seco" compound **7** could be accessed from a reassembly of advanced synthetic intermediates (Figure 4). The C11–C15 domain can be acylated with an appropriate C1 acid moiety to construct the C1–C15 ester linkage. The stereoselective formation of the C3 alcohol (in its native *S*-configuration) developed into a major challenge in our earlier efforts, especially in the epothilone F series.[11a] Extensive investigations revealed that the best yields were obtained from a chiral titanium-mediated *tert*-butyl acetate aldol reaction with aldehyde **6**, affording the correct C3 alcohol, *after* construction of the C6, C7, and C8 stereocenters.[20] For the synthesis of our cyclization precursor, acylation with acetic anhydride to generate the C15 acetate (vide infra), followed by an diastereoselective aldol reaction with aldehyde **6** would generate the target compound, with concomitant formation of the C3 stereocenter. Successful formation of

(15) Reviews: (a) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* **1995**, *28*, 446. (b) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18. (c) *Alkene Metathesis in Organic Chemistry*; Fürstner, A., Ed.; Springer: Berlin, 1998. (d) Fürstner, A. *Angew. Chem., Int. Ed.* **2000**, *39*, 3012. (e) Schrock, R. R. *Top. Organomet. Chem.* **1998**, *1*, 1.

(16) Xu, Z.; Johannes, C. W.; Salman, S. S.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1996**, *118*, 10926.

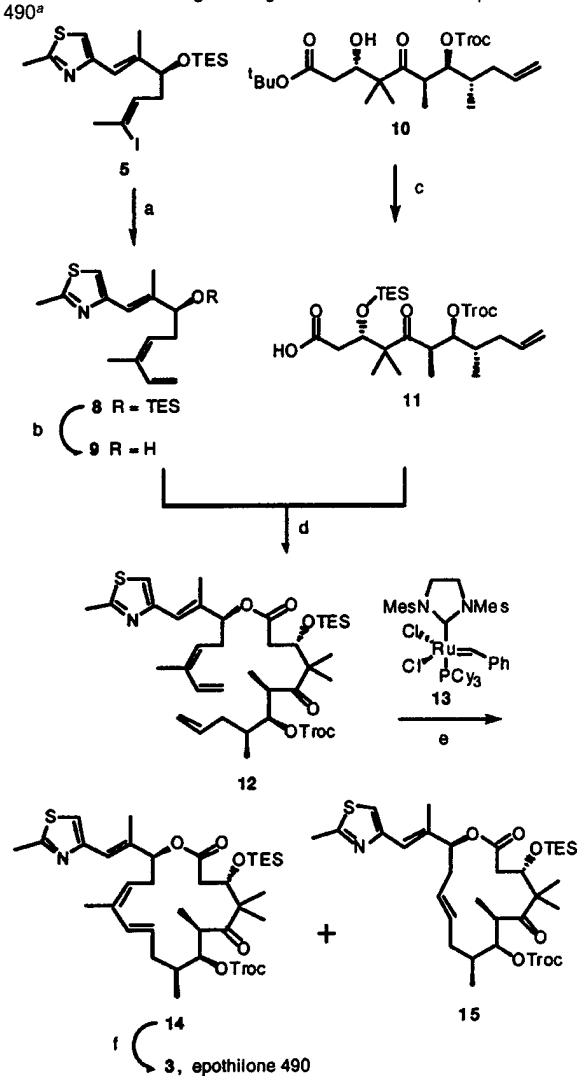
(17) (a) Garbaccio, R. M.; Danishefsky, S. J *Org. Lett.* **2000**, *2*, 3127. (b) Garbaccio, R. M.; Stachel, S. J.; Baeschlin, D. K.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 10903.

(18) (a) Cabrejas, L. M. M.; Rohrbach, S.; Wagner, D.; Kallen, J.; Zenke, G.; Wagner, J. *Angew Chem., Int. Ed.* **1999**, *38*, 2443. (b) Dvorak, C. A.; Schmitz, W. D.; Poon, D. J.; Pryde D. C.; Lawson, J. P.; Amos, R. A.; Meyers, A. I. *Angew. Chem., Int. Ed.* **2000**, *39*, 1664.

(19) Chappell, M. D.; Stachel, S. J.; Lee, C. B.; Danishefsky, S. J. *Org. Lett.* **2000**, *2*, 1633.

(20) Wu, Z.; Zhang, F.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2000**, *39*, 4505.

Scheme 1. Initial Ring-Closing Metathesis Route to Epothilone 490[a]



[a] Reagents and conditions: (a) $Pd_2(dba)_3$, CH_2=$CHSnBu_3$, PPh_3, DMF, 50 °C, 96%; (b) TBAF, THF, 0 °C, 92%; (c) TESOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to rt, 92%; (d) EDCI, DMAP, CH_2Cl_2, 0 °C to rt, 76%; (e) **13** (10 mol %), CH_2Cl_2 (0.002 M), 35 °C, 50% (**14**:**15** 3:1); (f) Zn, THF, AcOH, 86%; HF·pyr, THF, 0 °C, 90%.

the C3 (*S*)-alcohol late in the synthesis would obviate potential pitfalls in the construction of the C6−C8 stereotriad. With these design elements in mind, we embarked first upon the total synthesis of epothilone 490.

Given our quest for practicality, we insisted on convergent solutions to accomplish the C1−C2 interpolation and the creation of the diene functionality. In the event, Stille coupling[21] of **5** with vinyl *n*-tributyltin afforded **8** (Scheme 1). Cleavage of the silyl-protecting group afforded **9**. Our initial approach commenced with EDCI/DMAP-mediated esterification of the resulting allylic alcohol **9** with the C1 acid fragment **11**, obtained by deprotection of known *tert*-butyl ester **12**.[11b] This reaction yielded the cyclization precursor, triene **12**. Exposure of **12** to the RCM reaction with the second-generation ruthenium metathesis catalyst **13**[22] in methylene chloride gave a mixture of two compounds in a 3:1 ratio, with a total yield of 50%.[23] The major component of the product mixture was identified as the desired *trans*-substituted diene product **14**, along with the 14-membered macrolide **15** as a minor product, seemingly arising from a metathesis reaction involving the internal 12,13-olefin. Deprotection of the Troc and silyl groups *led to fully synthetic epothilone 490 (3), identical in all respects to an authentic sample*. The formation of the *E*-10,11-double bond was highly stereoselective and helped to confirm the stereochemistry of epothilone 490 to be as shown.

Following a similar series of reactions, we proceeded to synthesize the 21-hydroxyl variant of the new compound, the 10,11-dehydro version of desoxyepothilone F, compound **4**. Starting with the known Troc-protected 21-hydroxy vinyl iodide **16**,[11a] Stille coupling gave diene **17** (Scheme 2). Deprotection of the silyl group followed by esterification and RCM afforded **20**. Deprotection of the Troc and triethylsilyl groups afforded 21-hydroxy diene **4**.

A Surprising Substrate Effect on RCM Yield. Although, our initial foray into a new RCM manifold afforded a moderate yield of the macrolide, we were pleased to observe only the desired *E*-olefin in the reaction mixture. Examination of the sequence of steps that led to the construction of the cyclization precursor suggested a different order of conjoining the fragments in fewer total steps. Since the C3 (*S*)-stereocenter is constructed by a chiral titanium-mediated acetate aldol reaction,[20] we decided to attempt this reaction at a late stage, *with the entire O-alkyl fragment serving as part of the chiral nucleophile as its C15 acetate*. In this context, the allylic alcohol **9** was acylated to obtain the desired acetate **21** (Scheme 3). Following the protocol of Duthaler,[24] the lithium enolate of **21** was treated with the chiral titanium reagent to generate the chiral titanium enolate. Addition of aldehyde **6** afforded the desired aldol product, **22**, *as a single diastereomer*.[25]

Mindful of the fact that the newer ruthenium metathesis catalysts are tolerant of a wide variety of functional groups, we decided to attempt an RCM reaction on **22**, without protection of the C3 alcohol moiety. Treatment of **22** with catalyst **13** afforded the desired product in 41% yield, *with none of the 14-membered macrolide being observed*. Deprotection of the C7 Troc-protecting group in the usual way afforded epothilone 490.

The change in ratios of the 16- and 14-membered macrolide rings upon deprotection of the C3 alcohol suggested a surprising substrate effect on the macrocyclization step. We began to wonder about the potential effect of deblocking the C7 alcohol on the metathesis reaction as well. Therefore, we decided to perform a series of RCM reactions in which we varied the protection status of the C3 and the C7 alcohols in all of the possible combinations (Table 1). The results were indeed quite dependent on the presence of the protecting groups. The 14-membered macrolide was observed only when the substrate was fully protected. More importantly, *the yield of the reaction*

(21) Farina, V.; Krishnamurthy, V.; Scott, W. J. *Org. React.* **1997**, *50*, 1.

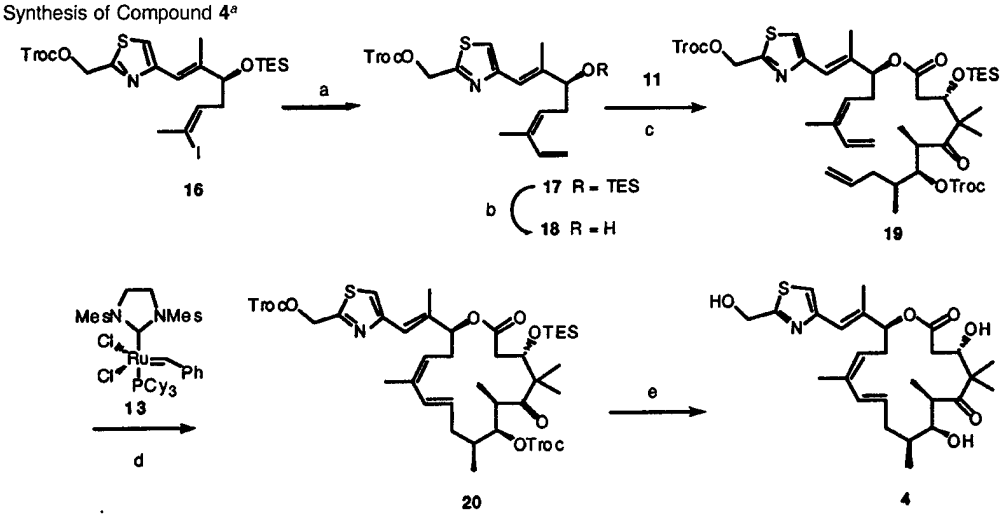
(22) Initial report: Scholl, M.; Trnka, T. M.; Morgan, J. P.; Grubbs, R. H. *Tetrahedron Lett.* **1999**, *40*, 2247.

(23) No reaction was observed with the first-generation bis(cyclohexyl)ruthenium Grubbs catalyst, while treatment with the Schrock molybdenum catalyst led to decomposition of the starting material.

(24) Duthaler, R. O.; Herold, P.; Lottenbach, W.; Oretle, K.; Reidiker, M. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 495.

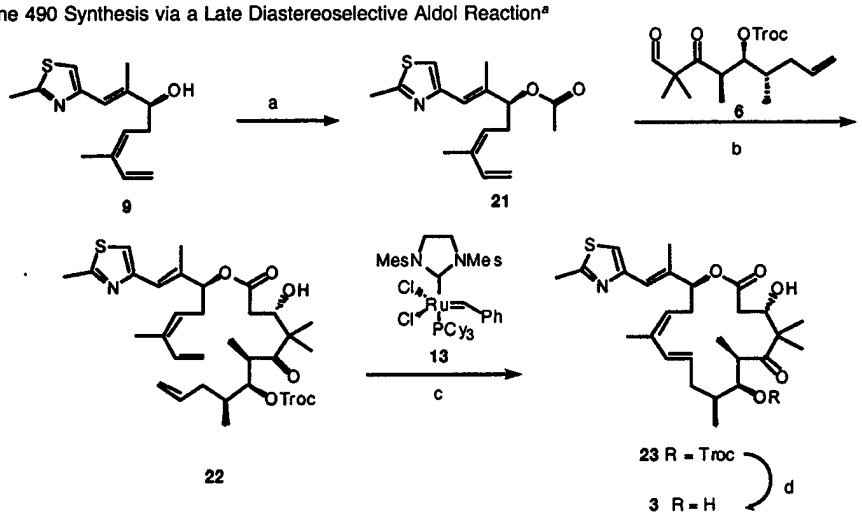
(25) The identity of the product was verified by treatment with TESCl to generate the C3 TES ether, which was identical to **12**, as determined by ^1H and ^{13}C NMR and optical rotation. Furthermore, **22** was converted to epothilone 490, verifying the (*S*)-stereochemistry at C3.

Scheme 2. Synthesis of Compound **4**[a]



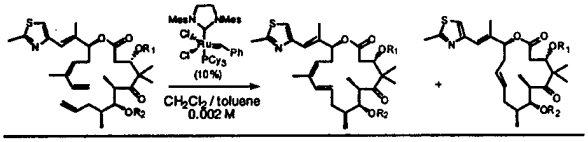
[a] Reagents and conditions: (a) $Pd_2(dba)_3$, $CH_2{=}CHSnBu_3$, PPh_3, DMF, 78%; (b) AcOH, THF, H_2O, 89%; (c) EDCI, DMAP, CH_2Cl_2, 0 °C to rt, 88%; (d) **13** (10 mol %), CH_2Cl_2 (0.002 M), 35 °C, 40%; (e) Zn, THF, AcOH, 70%; HF·pyr, THF, 0 °C, 80%.

Scheme 3. Epothilone 490 Synthesis via a Late Diastereoselective Aldol Reaction[a]



[a] Reagents and conditions: (a) Ac_2O, DMAP, Et_3N, CH_2Cl_2, 98%; (b) LDA, Et_2O, −78 °C, then $CpTiCl(OR)_2$ (R = 1,2:5,6-di-*O*-isopropylidine-α-L-glucofuranos-3-*O*-yl), −78 °C to −30 °C, then **6**, −78 °C, 85%; (c) **13** (10 mol %), CH_2Cl_2 (0.002 M), 35 °C, 41%; (d) Zn, THF, AcOH, 86%.

Table 1. Effect of Alcohol Protection and Different Solvents on RCM Yield[a]



12, R_1 = TES, R_2 = Troc	35% / 58%[b]	15% / 6%[b]
22, R_1 = H, R_2 = Troc	41% / 57%	0% / 0%
24, R_1 = TES, R_2 = H	57% / n.d.[c]	0% / n.d.[c]
25, R_1 = H, R_2 = H	64% / 55%	0% / 0%

[a] Reactions in CH_2Cl_2 were run for 5.5 h at 35 °C, reactions in toluene for 25 min at 110 °C. [b] Done with 20 mol % catalyst at 0.0005 M dilution. [c] Not determined.

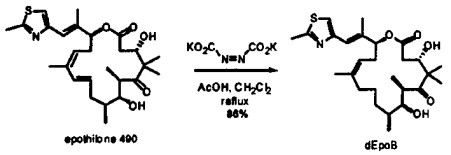
almost doubled upon use of a substrate where C7 is free. In fact, RCM of the fully deprotected substrate afforded the product epothilone 490 in 64% yield, with no observed *Z*-isomer of the C10−C11 olefin. This reaction represents the best yield obtained to date in construction of the epothilone scaffold in the B series with RCM, with no laborious separation of undesired olefin isomers involved in the purification process. Interestingly, when we carried out this same series of reactions in refluxing toluene, this substrate effect was diminished, with 55−58% yields observed across the various substrates.[26]

The origin of this substrate effect has not yet been determined. Intriguingly, we note that both the C3 and the C7 alcohols are β- to carbonyl groups, suggesting a possible contribution of intramolecular hydrogen bonding in imparting a degree of rigidity to the cyclization precursor.[27] Clearly, this effect does not seem to affect the reaction yield at higher temperature, in a less polar solvent.

(26) Toluene is a preferred solvent for scale-up processes; indeed, compound **22**, derived from the acetate aldol as shown in Scheme 3, was successfully subjected to metathesis conditions at 1 mmol scale in toluene at 110 °C as a proof of principle experiment. See Supporting Information for details. We thank Dr. Kana Yamamoto for suggesting the use of refluxing toluene conditions in these reactions

(27) For examples and discussion of similar protecting-group effects on RCM reactions in the synthesis of salicylihalamides, see: (a) Fürstner, A.; Thiel, O.; Blanda, G. *Org. Lett.* **2000**, *2*, 3731. (b) Fürstner, A.; Dierkes, T.; Thiel, O.; Blanda, G. *Chem. Eur. J.* **2001**, *7*, 5286 and references therein.

Scheme 4. Diimide Reduction of 10,11-Olefin: New Synthesis of dEpoB



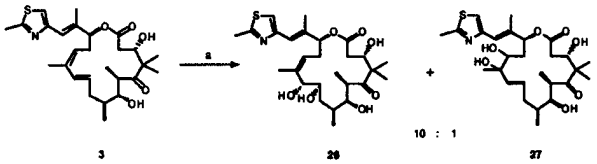
Selective Diimide Reduction of 10,11-Olefin: A New Route to dEpoB. The successful application of RCM to the synthesis of the diene epothilones of the 490 series led us to examine whether we could access our clinical candidate dEpoB by this newly described endgame. Attainment of this goal would involve a selective hydrogenation of the disubstituted C10−C11 *E*-olefin, in the presence of the trisubstituted C12−C13 *Z*-olefin and the "benzylic" trisubstituted C16−C17 olefin. A variety of metal-catalyzed and homogeneous hydrogenation conditions were examined, but they suffered from either over- or under-reduction.[28] Diimide-based reductions are known to be extremely sensitive to steric effects in distinguishing differentially substituted olefins.[29] Therefore, we turned our attention to diimide as a reducing agent to convert epothilone 490 to dEpoB. This goal was successfully accomplished by treatment of fully synthetic **3** with in situ generated diimide (86% yield, Scheme 4).

By focusing on a new section of the carbon skeleton for generation of an olefin, we have been able to successfully access the epothilone framework using an RCM−reduction protocol. Needless to say, the inspiration for this strategy was the isolation and identification of epothilone 490. During this process, we utilized the semipractical syntheses of advanced intermediates **5** and **6**, and fashioned the epothilone scaffold by a novel sequence of highly efficient reactions. The total synthesis reported herein is far more convergent and far less dependent on technically demanding reactions than the route which had already produced multigram quantities of clinical grade dEpoB. However, evaluation as to the feasibility of scale-up of the new route, in a plant context, has not been conducted at this writing.

Selective Functionalization of the 10,11-Olefin. The successful reduction reaction also indicated that selective functionalization of the newly generated C10−C11 olefin was feasible to enable a SAR profile of that sector of epothilones. Therefore, we report on the synthesis and preliminary evaluation of some novel epothilones available via epothilone 490. We subjected dienes in this series to dihydroxylation, epoxidation, and cyclopropanation conditions. Treatment of **3** with catalytic osmium tetroxide in the presence of NMO resulted in the formation of a 10:1 mixture, where the major product was identified as **26** (Scheme 5). The minor product arises from the dihydroxylation of the 12,13-olefin.

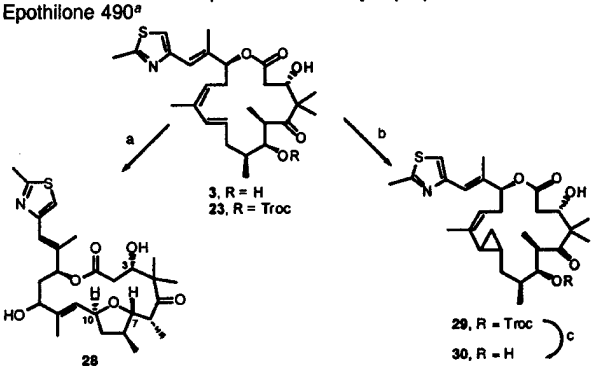
The stereochemistry at C10 and C11 of **26** was determined by X-ray crystallography, as depicted in Figure 5a. Inspection of a Macromodel-derived minimized (**MM2**) structure of epothilone 490, Figure 5b, shows that the "external" face of the 10−11 olefin is more available to reagents. This model

Scheme 5. Selective Dihydroxylation of 10,11-Olefin with OsO_4[a]



[a] Reagents and conditions: (a) OsO_4 (0.2 equiv), NMO (1.0 equiv), acetone:H_2O (9:1), −25 °C, 68%.

Scheme 6. Selective Epoxidation and Cyclopropanation of Epothilone 490[a]



[a] Reagents and conditions: (a) **3**, DMDO, CH_2Cl_2, −78 °C − rt, silica gel, 47%; (d) **23**, CH_2N_2, $Pd(OAc)_2$, Et_2O, 0 °C, 35%; (c) Zn, THF, AcOH, sonication, 85%.

suggests a rationalization of the product stereochemistry we observe in the dihydroxylation reaction.

Interestingly, exposure of **3** to the action of 2,2′-dimethyldioxirane, with the intent of generating an epoxide, gave rise to tetrahydrofuran-containing macrocycle **28** upon silica gel purification.[30] Compound **28** arises from epoxidation of the 12,13-olefin and S_N2'-type participation of the C-7 hydroxyl group (Scheme 6). Finally, treatment of **23** with diazomethane in the presence of $Pd(OAc)_2$,[31] followed by deprotection, afforded the vinyl cyclopropane **30**.[32]

The new analogues obtained from epothilone 490 exhibited a range of in vitro cytotoxities[9] and microtubule stabilizing ability,[33] as shown in Table 2. Indeed, the microtubule stabilizing ability closely parallels the observed cytotoxicity data.

The impressive cell growth inhibition exhibited by epothilone 490 across a range of various drug-resistant tumors led us to determine its efficacy in an in vivo setting, in nude mice bearing human tumor xenografts. Fortunately, our straightforward synthesis of **3** allowed us to indulge these interests. To our surprise, epothilone 490 did not demonstrate any meaningful inhibitory effect on the growth of the implanted tumors, as compared to control mice (data not shown). These data were surprising given the favorable prognosis based on in vitro protocols.

In addressing this problem, we recalled that dEpoB itself evidenced a worrisome bioinstability in murine plasma. However, it had much longer half-lives in higher organisms,

(28) Attempted conditions: H_2, Pd/C; H_2, Rh/Al_2O_3; H_2, Wilkinson's catalyst; H_2, PtO_2 (Adam's catalyst); H_2, Crabtree's catalyst.

(29) (a) Corey, E. J.; Mock, W. L.; Pasto, D. J. *Tetrahedron Lett.* **1961**, 347. (b) Pasto, D. J.; Taylor, R. T. *Org. React.* **1991**, *40*. 91. Importantly, J. D. White and co-workers have previously reported the reduction of a C9−C10 olefin during their synthesis of the epothilones with diimide, see ref 6k.

(30) The stereochemistry of macrocycle **28** was assigned on the basis of the analysis of 2D COSY and NOESY spectra, assuming that all the existing stereochemistry remained untouched under the mild reaction conditions.

(31) Denmark, S. E.; Stavenger, R. A.; Faucher, A.-M.; Edwards, J. P. *J. Org. Chem.* **1997**, *62*, 3375 and references therein.

(32) Stereochemistry of the new cyclopropane ring is undetermined at this writing. The 12-desmethyl version of this cyclopropyl analogue has recently been reported at the ACS National Meeting in Orlando, April 2002: Pabba, P. K.; Taylor, R. E. *Abstr. Pap. Am. Chem. Soc.* **2002**, *223*, 436-Orgn.

(33) Gaskin, F.; Cantor, C. R.; Shelanski, M. L. *J. Mol. Biol.* **1974**, *89*, 737.

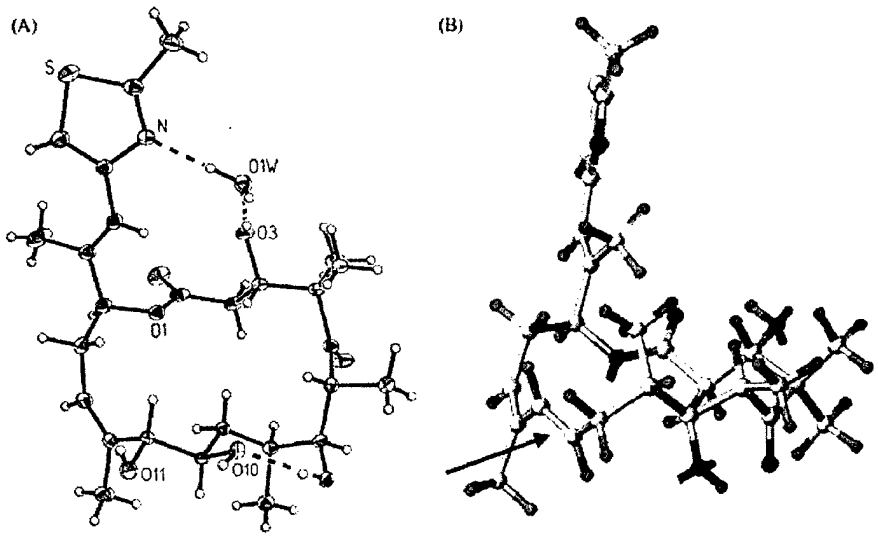


Figure 5. (a) X-ray structure of **26**, showing the stereochemistry of the dihydroxylation product to be the "external" diol. The new oxygens at C10 and C11 are in red. (b) Macromodel-derived minimized conformation of epothilone 490, demonstrating the easier access to the "external" face of the 10,11-olefin (marked by arrow) to reagents.

Table 2. In Vitro Cytotoxicities (IC_{50}) with Tumor Cell Lines[a] and Microtubule Binding

cmpd	CCRF-CEM (μM)	CCRF-CEM/$_{VBL100}$ (μM)	CCRF-CEF/$_{VM1}$ (μM)	CCRF-CEM/$_{Taxol}$ (μM)	% tubulin binding[b]
1 (dEpoB)	0.011	0.015	0.016	0.007	100
3	0.025	0.091	0.035	0.032	89
4	0.030	0.202	0.061	0.051	77
26	1.001	99.0	2.35	16.76	31
28	0.761	8.76	n.d.[c]	4.24	inactive
30	0.077	0.141	n.d.[c]	n.d.[c]	84
Taxol	0.0021	0.827	0.003	0.081	n.d.[c]
vinblastine	0.0008	0.122	0.0014	0.018	n.d.[c]

[a] XTT assay following 72-h inhibition. CCRF-CEM is a human T-cell acute lymphoblastic leukemia cell line. The CCRF-CEM/$_{VBL100}$, CCRF-CEM/$_{VM1}$, and CCRF-CEM/$_{Taxol}$ cell lines all overexpress P-glycoprotein and display a multidrug resistance phenotype to MDR-associated oncolytics.[9] [b] Formation of microtubules in the presence of the compounds. Microtubules formed in the presence of dEpoB is defined as 100%.[34] [c] Not determined.

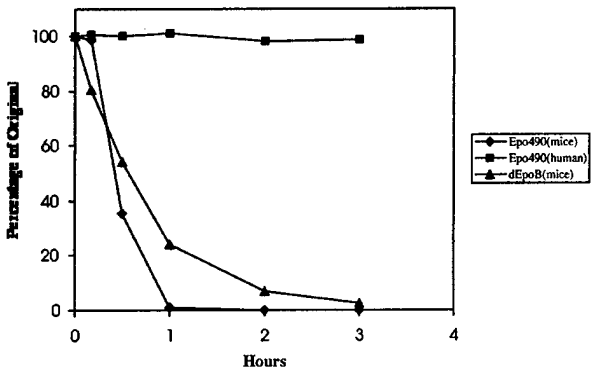


Figure 6. Plasma stability of epothilone 490 and dEpoB in nude mouse and human plasma (see ref 9 for details).

including humans.[9] This trend has been ascribed to higher esterase levels in rodents. The failure of epothilone 490 in our murine xenograft assay, in contrast to its excellent cell-culture inhibitory data, suggested that the pharmacokinetic properties of **3** be evaluated.

Indeed, on exposure of **1** and **3** to murine plasma, the biodegradation of epothilone 490 was even faster than that of dEpoB (Figure 6). Thus, while the murine pharmacokinetics of **1** are far from optimal, drug levels are adequate for major reduction and elimination of murine tumor burden. By contrast, the murine stability of **3** is so poor as to vitiate the potential benefits of the drug. *Encouragingly, the same drug remained essentially unchanged over 3 h in human plasma.*

Conclusions

The development of a clinically useful complex natural product demands easy access to vast quantities of purified material. Traditionally, isolation or fermentation-based methods have been the sole supplier of either the final product or of advanced intermediates which could be easily transformed to the desired drug by semisynthetic means. For example, the success of Taxol as a clinical candidate against solid tumors has been totally contingent upon the ability to procure the final product via semisynthesis from a readily accessible baccatin precursor.[35]

In this regard, *total* chemical synthesis of clinically useful complex natural products has been a much investigated but less productive tool. However, with the rapid development of efficient reaction processes, it could well be possible to gain

(34) See ref 6b for experimental details.
(35) (a) Denis, J. N.; Greene, A. E.; Guenard, D.; Guerittevoegelein, F.; Mangatal, L.; Potier, P. *J. Am. Chem. Soc.* **1988**, *110*, 5917. (b) Holton, R. A.; Liu, J. W. H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2475 and references therein.

rapid access to complex natural products by total synthesis. Our odyssey in the study and development of the epothilone-based family of anticancer agents is testimony to the power of chemical synthesis in supplying multigram quantities of these natural polyketides for preclinical and clinical evaluations of efficacy. In this report, we returned to our early attempts at fashioning the macrolide ring of the epothilones by ring-closing metathesis-based processes, which had been plagued by poor stereocontrol in the past. The identification of a series of natural epothilones with a new olefin at the C10−C11 position, and the development of more reactive metathesis catalysts prompted us to reexamine the utility of this reaction for generation of the epothilone macrocycle.

Herein, we described a construction of the epothilones with ring-closing metathesis. For purposes of greater synthetic convergency, we fashioned the C3 (*S*)-alcohol late in the synthesis, using a chiral titanium-mediated aldol reaction with the entire *O*-alkyl fragment as its C15 acetate as the enolate component. Examination of the effects of protecting groups on the RCM process demonstrated an intriguing substrate effect on reaction yield. Selective diimide reduction of the new olefin yielded desoxyepothilone B, our current clinical candidate, *validating the utility of this new RCM−reduction protocol in generating the epothilone framework.* The beginnings of charting the chemistry-based possibilities for analogue synthesis with **3** well in hand are described. Also described is the surprisingly poor in vivo performance of epothilone 490 in xenografts. This outcome was traceable to unfavorable pharmacokinetic features of the drug in this particular species. *To the extent that plasma stability is predictive of pharmacokinetic performance, the prognosis for the effectiveness of 3 in humans is much more promising.* Parenthetically, this research points to exciting possibilities in drug discovery and refinement centered around organic synthesis in close liaison with in vivo pharmacology and pharmacokinetics.

Acknowledgment. This work was supported by the National Institutes of Health (CA-28824). Postdoctoral support is acknowledged by K.B. (U.S. Army Breast Cancer Research Program, DAMD-17-98-1-8155), H.L. (NIH Cancer Pharmacology Training Grant, T32-CA62948), J.T.N. (General Motors Cancer Research Scholar Program) and M.D.C. (NIH, 1 F32 GM19972-01). We thank Dr. George Sukenick and Ms. Sylvi Rusli (NMR Core Facility, CA-02848) for mass spectral analyses and Joseph Tanski (Columbia University) for X-ray diffraction analysis.

Supporting Information Available: Experimental details for preparation and spectral characteristics of **8**, **9**, **11**−**12**, **14**−**15**, **3**, **17**−**20**, **4**, and **21**−**23** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA0262333

Total Syntheses of [17]- and [18]Dehydrodesoxyepothilones B via a Concise Ring-Closing Metathesis-Based Strategy: Correlation of Ring Size with Biological Activity in the Epothilone Series

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Abstract

A convergent ring-closing metathesis strategy has been employed for the highly concise syntheses of 10,11-dehydro-13,14-[17]desoxyepothilone B ([17]ddEpoB) and 10,11-dehydro-14,15-[18]desoxyepothilone B ([18]ddEpoB), which are 17- and 18-membered ring homologs of 10,11-dehydro-12,13-desoxyepothilone B ([16]ddEpoB or epothilone

490). We have demonstrated that the ring-closing metathesis (RCM) provides [17]ddEpoB or [18]ddEpoB with a high level of stereocontrol in the generation of the desired olefin in the products. These analogs were evaluated for antitumor activity. The results from the *in vitro* assays revealed that the [17]ddEpoB analog is highly active against various tumor cell lines with a potency comparable to that of the [16]ddEpoB. This is the first example of a 17-membered ring macrolactone epothilone that has retained its antitumor activity. In contrast, the biological data revealed that [18]ddEpoB is significantly less active than either [17]ddEpoB or the parent [16]ddEpoB.

Introduction.

The promotion of mitotic arrest by microtubulin stabilization has been at the forefront of the search for new leads in cancer chemotherapy for the past decade.[1] The most well known microtublin stabilization agent is paclitaxel (Taxol), which is currently one of the front-line drugs used for the treatment of cancer.[2] Despite this success, Taxol® is not necessarily the ultimate drug due to its susceptibility to multidrug resistance and the formulation difficulties arising from its lack of solubility in aqueous media.[3] These limitations drive the search for novel microtubulin stabilization agents that possess the potent activity of taxol but have a better therapeutic profile as well as increased water solubility. In the past decade, several microtubulin-stabilization agents have been discovered and studied with respect to overcoming the inherent limitations of paclitaxel.[1] Of these, the epothilones have been recognized as primary alternatives to paclitaxel in the light of their greater water solubility and their potent activity against multi-drug resistant cancer lines (Figure 1).[4] To date, several total syntheses of the naturally occurring

epothilones have been accomplished.[5] Subsequently, the preparation of hundreds of analogs allowed for the establishment of a detailed map of the structure-activity relationships based on *in vitro* and *in vivo* assays.[6]

[Figure 1]

While chemical modifications have been reported for many positions on the epothilone macrolide framework, the effects of ring size with respect to cytotoxic activity have been only briefly studied. The syntheses of 14-, 15-, 17-, 18-membered ring analogs of epothilone A have been reported by Nicolaou and coworkers.[7] These analogs, with the exception of one, had relatively weak tubulin binding activity in comparison to the [16]epothilone A (**1a**). The only exception was the [18]desoxyepothilone A, which revealed slightly lower tubulin polymerization activity in comparison to [16]epothilone A (**1a**). To provide further insight into the correlation between ring size of the epothilones and their corresponding biological activity, we elected to synthesize the corresponding 17- and 18-membered ring homologs of [16]ddEpoB (**2e**) and evaluate their antitumor activity.

We have been actively involved in total syntheses and structure activity relationship studies of the epothilones for some years.[8] Our epothilone program has led to the development of [16]dEpoB (**2b**) as a drug candidate, currently in human trials. The high demand for substantial quantities of [16]dEpoB (**2b**) for earlier preclinical studies and the lack of in house access to fermentation-derived material in our research setting, prompted our efforts to access the epothilones by total synthesis. We have previously shown how we addressed and solved the challenges associated with devising a concise, modular and efficient route to [16]ddEpoB (**2e**), a synthetic precursor of [16]dEpoB (**2b**),

based on a ring-closing metathesis based strategy.[9] As previously reported, **2e**, is highly active against various tumor cell lines with a potency comparable to that of the [16]dEpoB (**2b**). More importantly, the availability of [16]ddEpoB (**2e**) provided an opportunity for the synthesis of many new analogs of dEpoB **2b** that could not have been prepared using earlier synthetic routes. The disclosure herein describes the total synthesis 17- and 18-membered ring homologs of **2e**. While these syntheses draw from the previously described approaches, a significant modification was required if we were to attain the flexibility to control the ring size of the macrolactone via late stage variations. Preliminary biological evaluations of the novel compositions synthesized are provided below.

A highly convergent strategy, related to that employed in the synthesis of [16]ddEpoB (**2e**)[9], was used. Accordingly, fragments of similar complexity served as key building blocks (Scheme 1). We envisioned that the acyl sector **6**,[9] could serve as the polypropionate domain and the alkyl sector **5a** or **5b** would be prepared in a few steps from a known intermediate.[9] The union of the two fragments **5a**(**5b**) and **6** would be initiated through an esterifciation and consumated via a subsequent ring-closing metathesis. Finally, cleavage of the protecting groups would provide the desired 17- and 18-membered ring homologs (**3a** and **3b**) of **2e**.

[Scheme 1]

Results and Discussion.

The synthesis of the 17- and 18-membered ring homologs commenced with the conversion of the previously reported vinyl iodide **7**[10] to the corresponding 1,4-diene **5a** and 1,5-diene **5b** (Scheme 2). Reaction of vinyl iodide **7**, with allyltributyltin under Stille

conditions, afforded the desired 1,4-diene **8** in 92% yield. Correspondingly, reaction of vinyl iodide **7** with butenylmagnesium bromide under the Tamao-Kumada-Corriu palladium(0) mediated coupling conditions[11] provided the desired 1,5-diene **9** in 75%. It seems likely that this reaction could be extended towards the synthesis of alternative unconjugated dienes, which could allow for the synthesis of even larger ring analogs. Finally, treatment of 1,4-diene **8** and 1,5-diene **9** with tetra-*n*-butylammonium fluoride accomplished deprotection of the secondary alcohol in high yield.

[Scheme 2]

Esterification of the resultant allylic alcohols **5a** and **5b** with C_1-C_{10} acid fragment **6** provided the corresponding RCM cyclization precursors in 61% (**4a**) and 67% (**4b**) yields, respectively (Scheme 3). The ring-closing metathesis reaction of 1,4-diene **4a** was then carried out using the second generation Grubbs catalyst[12] in methylene chloride, which provided, as in our earlier study,[9] exclusively the *trans* isomer **10a** in a yield of 58%. Using the same RCM reaction conditions with the 1,5-diene **4b** provided exclusively the *trans* isomer **10b** in 55% yield, along with recovered starting material. Finally, reductive cleavage of the 2,2,2-trichloroethoxycarbonyl protecting group with zinc and acetic acid followed by deprotection of triethylsilyl ether with HF-pyridine led to the [17]-and [18]ddEpoB (**3a** and **3b**).

[Scheme 3]

The fully synthetic [17]- and [18]ddEpoB have been evaluated against a variety of cell types to determine their antitumor potential. As shown in table 1, [17]ddEpoB (**3a**) exhibited high cytotoxic activity against a variety of sensitive and resistant tumor cell lines. Direct comparison of [17]ddEpoB (**3a**) with the previously reported [16]ddEpoB

(**2e**) indicates that the new compound possess comparable potency. In contrast, the data reveal that [18]ddEpoB (**3b**) is significantly less active than either [17]ddEpoB (**3a**) or [16]ddEpoB (**2e**).[7] Preliminary model studies have indicated that there is only a small difference between the overall configuration of [17]ddEpoB (**3a**) and [16]ddEpoB (**2e**), whereas there is a large difference between the overall configuration of [18]ddEpoB (**3b**) and [16]ddEpoB (**2e**). Thus, we suggest that the alterations in overall configuration in the case of [18]ddEpoB (**3b**) may have led to the distortion of the essential pharmacore and the reduction of antitumor activity.

[Table 1]

In summary, the key step, which controls the eventual ring size of the ultimate epothilone is a cross coupling of vinyl iodide under mediation by Pd(0). The total syntheses of [17]ddEpoB (**3a**) and [18]ddEpoB (**3b**) have been achieved using a strategy based on a convergent merger of two major fragments by an esterification and subsequent ring-closing metathesis.[13] Application of the second generation Grubbs catalyst in the ring-closing metathesis in the synthesis of [17]- and [18]ddEpoB (**3a** and **3b**) gave exclusively the *trans* olefinic isomer. It seems likely that the ring-closing metathesis strategy described herein can be extended toward the synthesis of higher ring homologs of the epothilones.

The *in vitro* tumor growth inhibition experiments demonstrated the new [17]ddEpoB (**3a**) analog possesses high *in vitro* antitumor activity, which is comparable to that of [16]ddEpoB (**2e**). This represents the first example of a 17-membered ring epothilone macrolide that has antitumor activity similar to the 16-membered natural products. The dramatic diminution in activity of [18]ddEpoB (**3b**) gives further support

towards the limited tolerance of the pharmacophore to distortion. Further investigations with [17]ddEpoB (**3a**) are currently underway.

Acknowledgments. This research was supported by the National Institutes of Health (grant numbers: S.J.D. (CA-28824), T.C.C. (CA-08748). Postdoctoral Fellowship support is gratefully acknowledged by: A.R. (NIH Cancer Pharmacology Training Grant, T32-CA62948), K.B. (US Army Breast Cancer Research Program, DAMD-17-98-1-8155), J.T.N. (General Motors Cancer Research Scholar Program). The authors wish to thank Dr. George Sukenick (NMR Core Facility, Sloan-Kettering Institute, and CA-08748) for mass spectral and NMR analysis.

Supporting Information Available: ^{1}H and ^{13}C NMR Spectra for all characterized compounds are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

1 Lu, M. C., Foye, W. O. (Eds.), Cancer Chemotherapeutic Agents, American Chemical Society, Washington D. C., 1995, pp. 345-368.

2 (a) Rowinsky, E. K. *Annu. Rev. Med.* **1997**, *48*, 353-374. (b) Nicolaou, K. C.; Dai, W.-M.; Guy, R. K. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 15.

3 (a) Bollag, D. M.; McQueney, P. A.; Zhu, J.; Hensens, O.; Koupal, L.; Leisch, J.; Goetz, M.; Lazarides, E.; Woods, C. M. *Cancer Res.* **1995**, *55*, 2325; (b) Kowalski, R. J.; Terhaar, E.; Longley, R. E.; Gunasekera, S. P.; Lin, C. M.; Day, B. V.; Hamel, E. *Mol. Biol. Cell* **1995**, *6*, 2137; (c) Rowinsky, E. K.; Eisenhauer, E. A.; Chaudhry, V.; Arbuck, S. G.; Donehawer, R. C. *Semin. Oncol.* **1993**, *20*, 1; (d) Fletcher, B. S.; Kujubadu, D. A.; Perrin, D. M.; Herschman, H. R. *J. Biol. Chem.* **1992**, *267*, 4338; (e) Tsuji, M.; Dubois, R. N. *Cell*, **1995**, *3*, 493; (f) Essayan, D. M.; Kagey-Sobotka, A.; Colarusso, P. J.; Lichtenstein, L. M.; Ozols, R. F.; King, E. D. *J. Allergy Clin. Immunol.I* **1996**, *97*, 42; (g) Giannakakou, P.; Sackett, D. L.; Kang, Y.-K.; Zhan, Z.; Buters, J. T.; Fojo, T.; Poruchynsky, M. S. *J. Biol. Chem.* **1997**, *272*, 17118 and references therein.

4 Höfle, G. H.; Bedorf, N.; Steinmetz, H.; Schomburg, D.; Gerth, K.; Reichenbach, H. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1567.

5 (a) Balog, A.; Meng, D. F.; Kamenecka, T.; Bertinato, P.; Su, D.-S.; Sorensen, E. J.; Danishefsky, S. J. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2801; (b) Su, D.-S.; Meng, D. F.; Bertinato, P.; Balog, A., Sorensen; E. J.; Danishefsky, S. J.; Zheng, Y. H.; Chou, T. C.; He, L. F.; Horwitz, S. B. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 757; (c) Meng, D. F.; Bertinato, P.; Balog, A.; Su, D.-S.; Kamenecka, T.; Sorensen, E. J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 10073. For initial reports of other epothilone syntheses, see: (d) Yang, Z.; He, Y.; Vourloumis, D.; Vallberg, H.; Nicolaou, K. C. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 166; (e) Nicolaou, K. C.; Sarabia, F.; Ninkovic, S.; Yang, Z. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 525; (f) Nicolaou, K. C.; Winssinger, N.; Pastor, J.; Nincovic, S.; Sarabia, F.; He, Y.; Vourloumis, D.; Yang, Z.; Li, T.; Giannakakou, P.; Hamel, E. *Nature* **1997**, *387*, 268; (g) Schinzer, D.; Limberg, A.; Bauer, A; Bohm, O. M.; Cordes, M. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 523; (h) May, S. A.; Greico, P. A. *Chem. Commun.* **1998**, 1597; (i) Sawada, D.; Shibasaki, M. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 209; (j) Martin, H. J.; Drescher, M.; Mulzer, J. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 581; (k) White, J. D.; Carter, R. G.; Sundermann, K. F. *J. Org. Chem.* **1999**, *64*, 684; (l) Zhu, B.; Panek, J. S. *Org. Lett.* **2000**, *2*, 2575, (m) Bode, J. W.; Carreira, E. M. *J. Am. Chem. Soc.* **2001**, *123*, 3611; (n) Fürstner, A.; Mathes, C.; Grela, K. *Chem. Commun.* **2001**, 1057; (o) Taylor, R. E.; Chen, Y. *Org. Lett.* **2001**, *3*, 2221; (p) Valluri, M.; Hindupur, R. M.; Bijoy, P.; Labadie, G.; Jung, J. C.; Avery, M. A. *Org. Lett.* **2001**, *3*, 3607; (q) Ermolenko, M. S.; Potier, P. *Tetrahedron Lett.* **2002**, *43*, 2895.

6 (a) Florsheimer, A., Altmann, K. H. *Expert Opin. Ther. Patents* **2001**, *11*, 951; (b) Nicolaou, K. C., Roschangar, F., Vourloumis, D. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2014-2045; (c) Chou, T. C.; Zhang, X. G.; Harris, C. R.; Kuduk, S. D.; Balog, A.; Savin, K. A.; Bertino, J. R.; Danishefky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15798; (d) Chou, T. C.; Zhang, X. G.; Balog, A.; Su, D.; Meng, D. F.; Savin, K.; Bertino, J. R.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9642.

7 Nicolaou, K. C.; Sarabia, F.; Ninkovic, S.; Ray, M., Finlay, V., Boddy, C. N. C. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 81.

8 Chou, T. C.; O'Connor, O. A.; Tong, W. P.; Guan, Y.; Zhang, Z.-G.; Stachel, S. J.; Lee, C.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8113.

9 Biswas, K; Lin, H.; Njardarson, J.T.; Chappell, M.D., Chou, T.C., Guan, Y.; Tong, W. P., He, L.; Horwitz, S.B., Danishefsky, S.J. *J. Am. Chem. Soc.* **2002**, In Press.

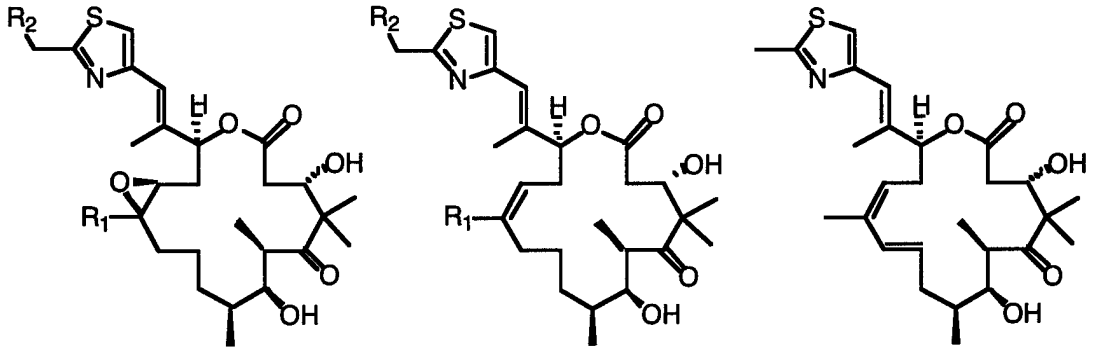
10 Chappell, M. D.; Stachel, S. J.; Lee, C. B.; Danishefsky, S. J. *Org. Lett.* **2000**, *2*, 1633.

11 (a) Tamao, K., Sumitani, K., Kumada, M. *J. Am. Chem. Soc.* **1972**, *94*, 4374; (b) Corriu, R. J., Masse, J. P. *Chem. Comm.* **1972**, 144.

12 Reviews: (a) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* **1995**, *28*, 446; (b) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18; (c) *Alkene Metathesis in Organic Chemistry* Ed.: Fürstner, A.; Springer, Berlin, **1998**; (d)

Fürstner, A. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 3012; (e) Schrock, R. R. *Top. Organomet. Chem.* **1998**, *1*, 1.

13 See ref. [9] and Sinha, S. C.; Sun, J. *Angew. Chem. Int. Ed. Engl.* **2002**, *41*, 1381.

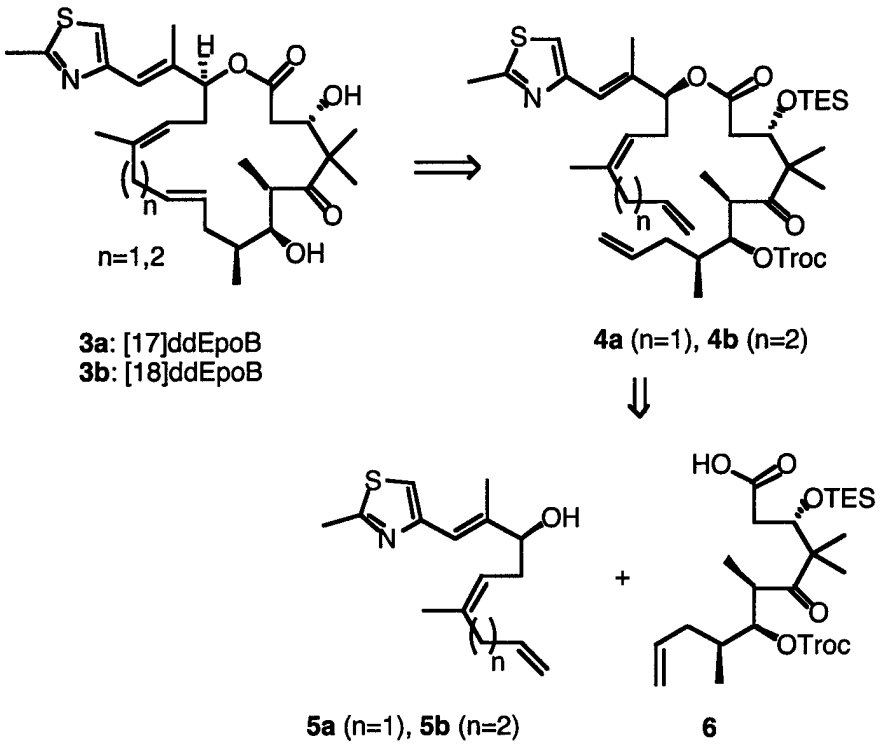


1 **2** **2e**

1a R_1 = H, R_2 = H, Epothilone A
1b R_1 = CH_3, R_2 = H, Epothilone B
1c R_1 = H, R_2 = OH, Epothilone E
1d R_1 = CH_3, R_2 = OH, Epothilone F

2a R_1 = H, R_2 = H, Epothilone C
2b R_1 = CH_3, R_2 = H, Epothilone D ([16]dEpoB)
2c R_1 = H, R_2 = OH, Desoxyepothilone E
2d R_1 = CH_3, R_2 = OH, Desoxyepothilone F
2e 10,11-Dehydroepothilone D ([16]ddEpoB)

Figure 1. Structure of [16]Epothilones and [16]Desoxyepothilones



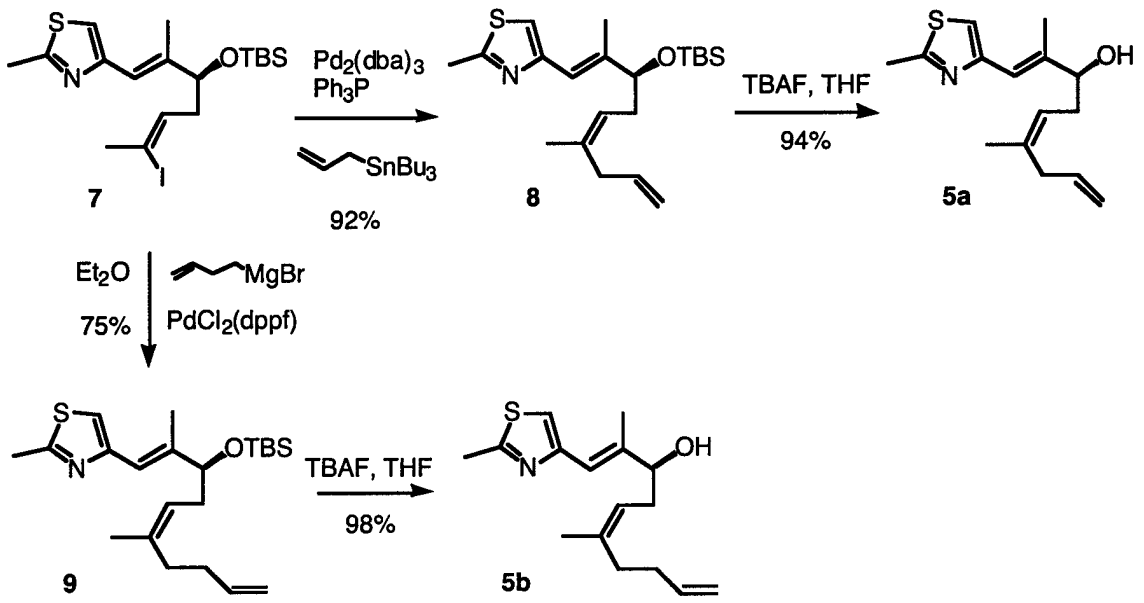
3a: [17]ddEpoB
3b: [18]ddEpoB

4a (n=1), **4b** (n=2)

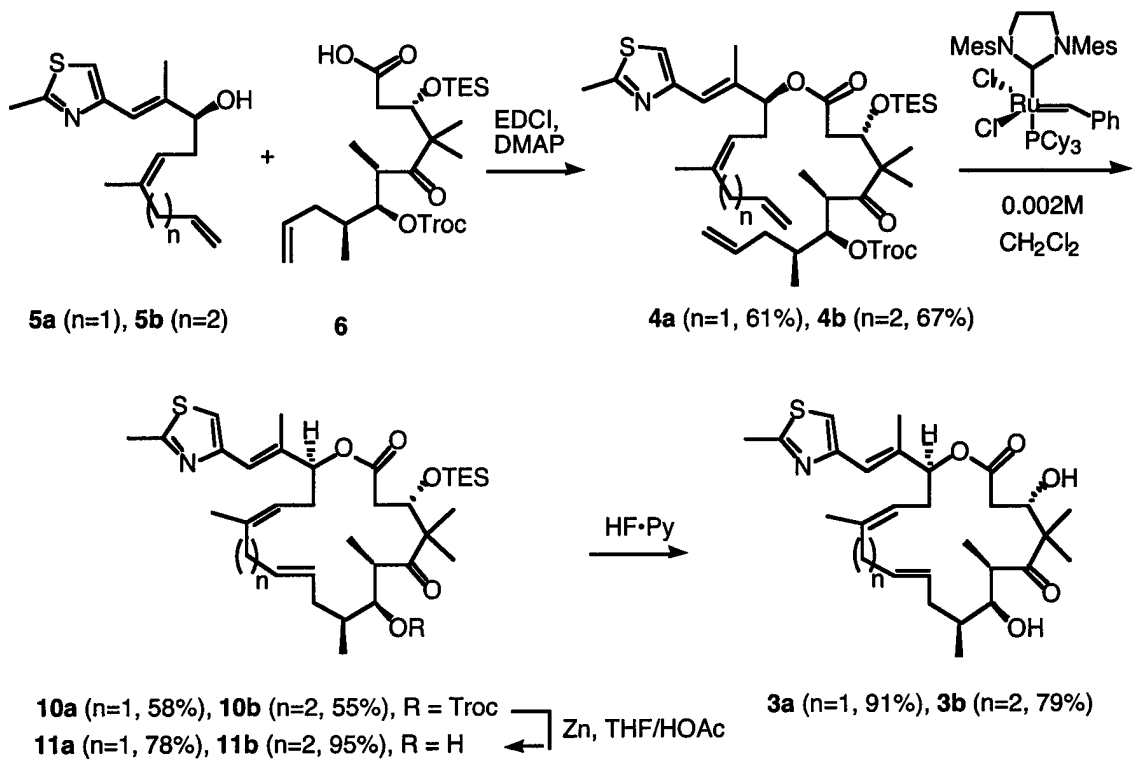
5a (n=1), **5b** (n=2)

6

Scheme 1. Application of RCM Strategy towards the Synthesis [17]- and [18]ddEpoB



Scheme 2. Preparation of left fragments **5a** and **5b**



Scheme 3. The Synthesis of [17]ddEpoB and [18]ddEpoB

Table 1. *In vitro* Cytotoxicities (IC_{50}) with tumor cell lines[a].

Tumor Cell Lines	IC_{50} (μM)[a]			
	[17]ddEpoB(**3a**)	[18]ddEpoB(**3b**)	[16]ddEpoB(**2e**)	dEpoB(**2b**)
CCRF-CEM	0.040	0.322	0.025	0.011
CCRF-CEM/VBL_{100}	0.126	0.870	0.091	0.015
CCRF-CEM/VM_1	0.055	ND	0.035	0.016
CCRF-CEM/Taxol	0.053	0.508	0.032	0.007

[a]XTT assay following 72 h inhibition. CCRF-CEM is a human T-cell acute lymphoblastic leukemia cell line. The CCRF-CEM/$_{VBL100}$, CCRF-CEM/$_{VM1}$ and CCRF- CEM/$_{Taxol}$ cell lines all overexpress P-glycoprotein and display a multidrug resistance phenotype to MDR associated oncolytics.[8]

On the Introduction of a Trifluoromethyl Substituent in the Epothilone Setting: Chemical Issues Related to Ring Forming Olefin Metathesis and Earliest Biological Findings

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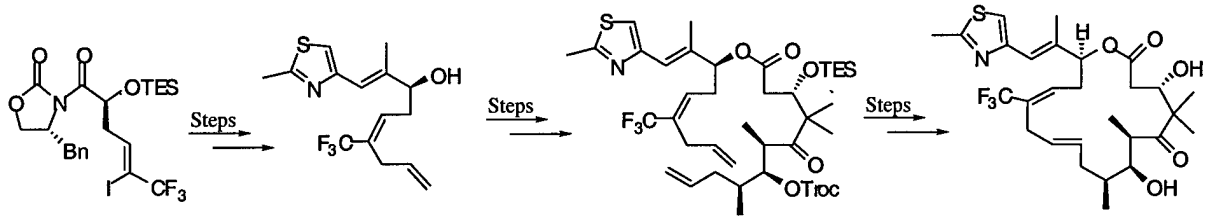


9 23 18 19

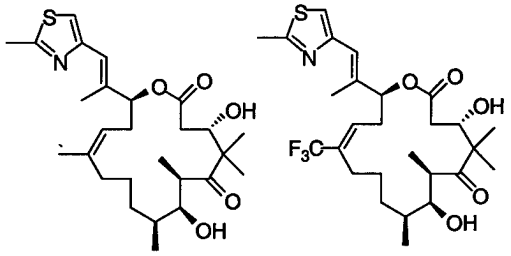
ABSTRACT

The disclosure herein describes the synthesis of 10,11-dehydro-13,14-desoxy-27-trifluoro-[17]epothilone B via a stereoselective ring-closing metathesis and provides early biological evaluation data pertinent to this compound.

In the past five years, the epothilones have emerged as promising new anticancer agents.[1] Human clinical trials seeking to assess issues of toxicity, optimal dosage, and likely

efficacy of several epothilones as drugs are well underway.[2] For instance, 12,13-desoxyepothilone B, initially developed in our laboratory via total synthesis, is now undergoing human clinical trials.[3] Given the massive interest in epothilones, it is not surprising that there has been a worldwide effort to synthesize new analogs, and to establish their SAR with a view to identifying and developing later generation agents for clinical evaluation.[4] Given the important role of fluorine susbtitution in enhancing pharmacokinetics and chemotheraputic indices of many medicinal agents,[5] it was natural to evaluate this type of structutal perturbation in the epothilone series. We initially targeted compound **2**, which is seen to correspond to a 26-trifluoroepothilone congener for synthesis and biological evaluation.

Figure 1. Selected Epothilone Analogs

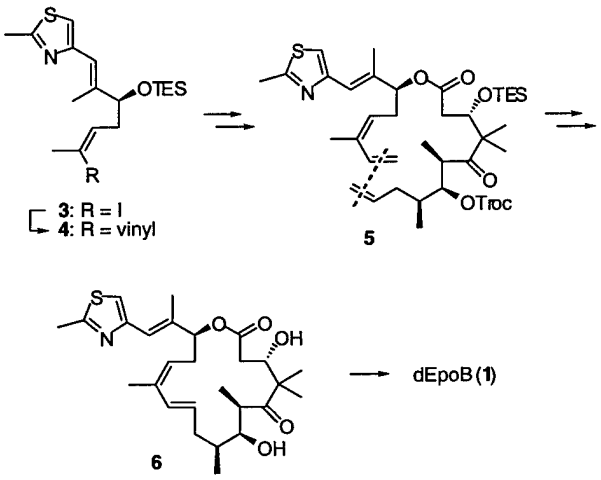


1: [16]dEpoB **2**: 26-F_3-[16]dEpoB

To reach compound **2**, we sought to take advantage of a highly convergent route recently reported from our laboratory for the synthesis of epothilone 490 (**6**, dehydrodeoxy Epo B)) en route to dEpoB (**1**, Scheme 1).[6] In that synthesis, we introduced a flanking vinyl group to compound **4** via a stereospecific Stille coupling of a vinyl iodide precursor **3** with tri-*n*-

butylvinylstannane. Ring closing metathesis followed by deprotection led to **6**, which was then transformed to dEpoB (**1**) via a regioselective diimide reduction.

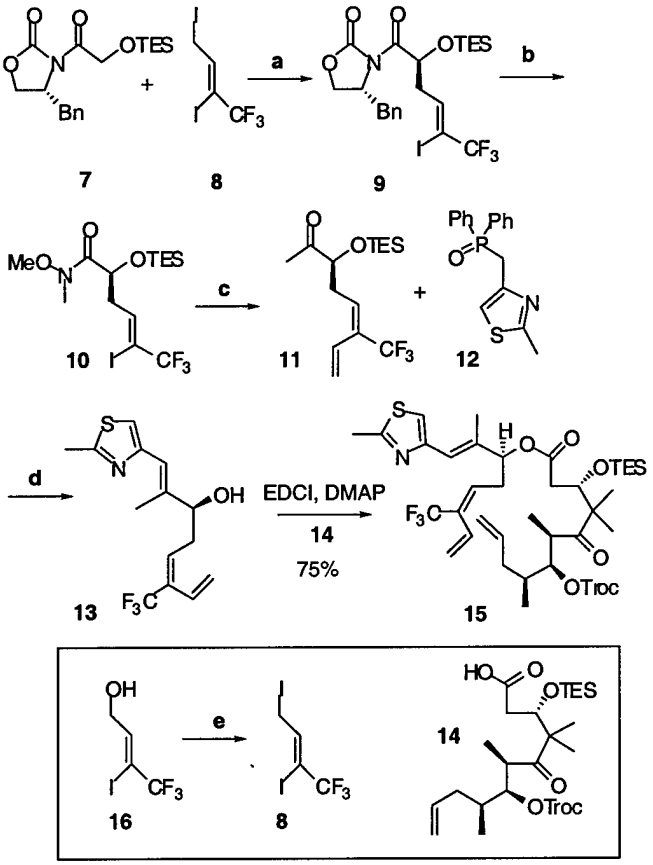
Scheme 1. Synthesis of Epothilone 490



Attention was first directed to the synthesis of **15** (Scheme 2). Alkylation of the previously reported lithium enolate of **7**[7] with iodide **8** (synthesized from the known alcohol **16**[8] using TMSI in methylene chloride) afforded **9** in 78% yield and high diastereoselectivity (>25:1 de). Compound **9** was advanced in three steps to **10** as shown. Attempts to accomplish addition of methylmagnesium bromide to the Weinreb amide linkage of **10**, failed to provide **11**. The breakdown of this reaction was attributed to the presence of the iodoalkene linkage. However we could accomplish our goal by changing the order of these two C-C bond forming steps. Thus, reaction of **10** with vinyltributyltin under Stille conditions could then be followed by addition of methyl Grignard reagent to give the desired ketone **11**. Condensation of ketone **11** with phosphine oxide **12**, followed by deprotection of the triethylsilyl ether, afforded fragment

13 in good yield. Esterification of the resulting **13** with C1-C10 acid fragment **14**[6], provided the desired **15**, in 75% yield (Scheme 2).

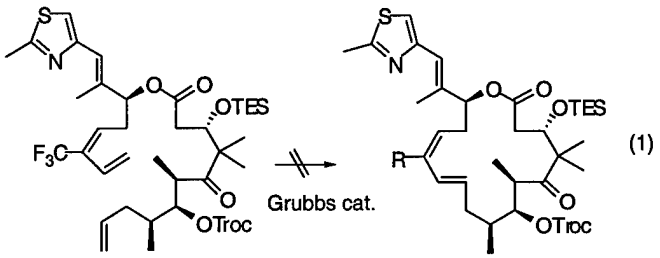
Scheme 2. Synthesis of the RCM precursor 15



(a) LHMDS, -78 °C, 78%; **(b)** i) HOAc:THF:H_2O (3:1:1); ii) CH_3ONHCH_3, $AlMe_3$; iii) TESCl, imidazole, DMF, 79% overall; **(c)** i) Vinyltributyltin, Pd(dba), DMF, 80 °C, 3h 43%; ii) MeMgBr, 0 °C, 94%; **(d)** i) *n*-BuLi, THF, -78 °C, 30 min., ii) **12**, -78 °C to rt, 81%; iii) HOAc:THF:H_2O (3:1:1), 94%; **(e)** TMSI, CH_2Cl_2, 0 °C, 92%

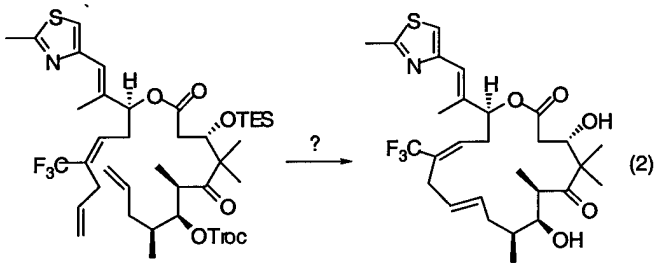
Unfortunately, attempts to carry out the ring-closing metathesis reaction of **15** using the second generation Grubbs catalyst[9] in methylene chloride led primarily to apparent dimerization of the starting material (Equation 1). Given the fact that the RCM works quite well

in the related setting of **5** → **6**, we naturally attributed the failure in the case of **15** to the presence of the trifluoromethyl group at C_{12}.



15 **17**

It was conjectured that the detrimental impact of the resident 26-trifluoro substitutent on the desired reaction, might be alleviated by adding a carbon spacer between the RCM reaction center and the trifluoromethyl group. Accordingly, we undertook a synthesis of **19** (Equation 2) via the ring-closing metathesis of **18**, which would present the trifluoromethyl group in the context of a 17-membered ring containing a shipped (1,4)diene.

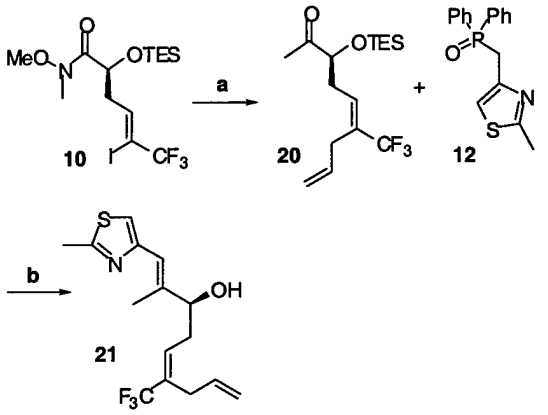


18 **19**

The synthesis program directed to **19** commenced with the preparation of compound **21**, which corresponds to the O-alkyl sector of our proposed RCM substrate (Scheme 3). We began with allylation of **10**, this time under radical reaction conditions as shown.[10] This conversion was

followed by reaction of the alkylated product with of methyl magnesium bromide, thus affording the required ketone **20**. Condensation of this compound with phosphine oxide **12**, followed by deprotection of the triethylsilyl ether function provided **21** in good yield.

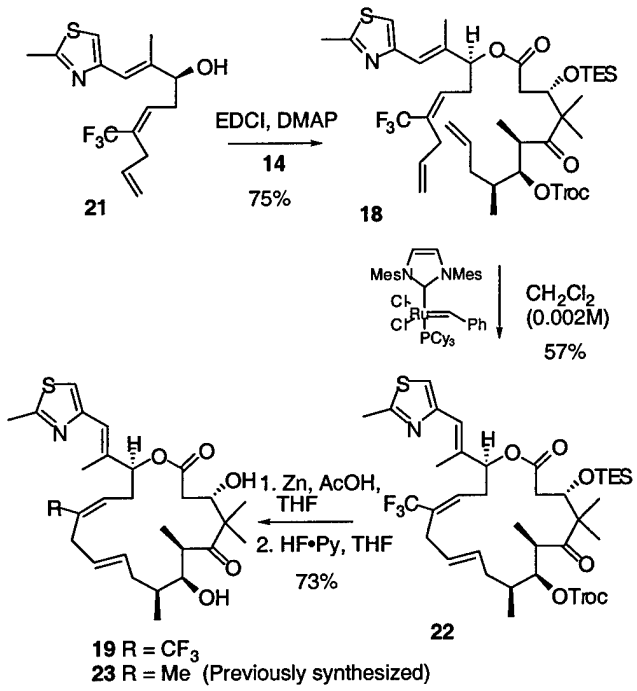
Scheme 3. Synthesis of the alcohol fragment 21



(a) i) Allyltributyltin, AIBN, Benzene, 80 °C, 3h 74%; ii) MeMgBr, 0 °C, 93%; **(b)** i) **12**, *n*-BuLi, THF, -78 °C, 30 min., ii) **22**, -78 °C to rt, 85%; iii) HOAc:THF:H_2O (3:1:1), 98%; **(c)** TMSI, CH_2Cl_2, 0 °C, 92%

Esterification of **21** with the C1-C10 acid fragment **14**, provided the proposed RCM precursor **18** in 75% yield (Scheme 4). Happily in this case, the ring-closing metathesis reaction of **18** could be accomplished using the second generation Grubbs catalyst in methylene chloride. As in the case of the conversion of **5**→**6**, the reaction provided exclusively the *trans* isomer **24** in 57% yield.[6] Finally, reductive cleavage of the trichloro ethoxycarbonyl protecting group with zinc and acetic acid, followed by deprotection of the TES ether with HF-pyridine, provided the desired **24** containing a trifluoromethyl function at C_{12}, albeit in the context of the 17-membered ring series.

Scheme 4. Synthesis 27-F_3-ddEpoB (19)



Synthetic **19** was evaluated as to its cytotoxic activity. As shown in Table 1, direct comparison of the previously reported [17]ddEpoB (**23**) with 27-F_3-[17]ddEpoB (**19**) indicated that the new perfluorinated compound possessed a comparably high cytotoxic potency. Though the trifluoromethyl isoteric substitution had little effect on the gross cytotoxic activity, *preliminary data from metabolic degradation studies in mouse plasma showed **19** to be notably more stable than is the parent **23**.*[11] Since pharmokinetic issues are likely to be critical in the actual use of any epothilone agent as a drug, we take this finding to be quite encouraging. We are pursuing new departures directed to the incorporation of trifluoromethyl substituents in various epothilone settings.

Table 1. *In vitro* Cytotoxicities (IC_{50}) with tumor cell lines[a]

Compound	CCRF-CEM (IC_{50} (μM)[a])	CCRF-CEM/ VBL (IC_{50} (μM)[a])
27-Tri-F-[17]ddEpoB (**19**)	0.068	0.191
[17]ddEpoB (**23**)	0.040	0.126
[16]ddEpoB (**6**)	0.020	0.068

[a]XTT assay following 72 h inhibition. CCRF-CEM is a human T-cell acute lymphoblastic leukemia cell line. The CCRF-CEM/$_{VBL100}$, CCRF-CEM/$_{VM1}$ and CCRF- CEM/$_{Taxol}$ cell lines all overexpress P-glycoprotein and display a multidrug resistance phenotype to MDR associated oncolytics.[5]

Acknowledgment. This research was supported by the National Institutes of Health (grant numbers: S.J.D. (CA-28824), T.C.C. (CA-08748). Postdoctoral Fellowship support is gratefully acknowledged by: A.R. (NIH Cancer Pharmacology Training Grant, T32-CA62948) and K.B. (US Army Breast Cancer Research Program, DAMD-17-98-1-8155). The authors wish to thank Dr. George Sukenick (NMR Core Facility, Sloan-Kettering Institute, and CA-08748) for mass spectral and NMR analysis.

References:

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(1) For extensive reviews in this field see: (a) Nicolaou, K. C.; Roschangar, F.; Vourloumis, D. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2015. (b) Harris, C. R.; Danishefsky, S. J. *J. Org. Chem.* **1999**, *64*, 8434.

(2) Chou, T. C.; O'Connor, O. A.; Tong, W. P.; Guan, Y.; Zhang, Z.-G.; Stachel, S. J.; Lee, C.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8113.

(3) For more information about clinical trials of dEpoB, visit: www.kosan.com.

(4) (a) Florsheimer, A., Altmann, K. H. *Expert Opin. Ther. Patents* **2001**, *11*, 951; (b) Nicolaou, K. C., Roschangar, F., Vourloumis, D. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2014-2045; (c) Chou, T. C.; Zhang, X. G.; Harris, C. R.; Kuduk, S. D.; Balog, A.; Savin, K. A.; Bertino, J. R.; Danishefky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15798; (d) Chou, T. C.; Zhang, X. G.; Balog, A.; Su, D.; Meng, D. F.; Savin, K.; Bertino, J. R.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9642.

(5) (a) Ojima, I.; Inoue, T.; Chakravarty, S.; *J. Fluorine Chem.* **1999**, *97*, (b) Newman, R. A.; Yang, J.; Finlay, M. R. V.; Cabral, F., Vourloumis, D.; Stephens, L. C.; Troncoso, P.; Wu, X.; Logothetis, C. J.; Nicolaou, K. C.; Navone, N. M. *Cancer Chemother. Pharmacol.* **2001**, *48*, 319-326.

(6) (a) Biswas, K.; Lin, H.; Njardarson, J. T.; Chappell, M.D., Chou, T.C., Guan, Y.; Tong, W. P., He, L.; Horwitz, S.B., Danishefsky, S.J. . *J. Am. Chem. Soc.* **2002**, J. Am. Chem. Soc.; (Article); 2002; 124(33); 9825-9832.. (b) Rivkin, A.; Njardarson, J. T.; Biswas, K; Chou, T.C.; Danishefsky, S. J. *J. Org. Chem.* **2002**, In Press.

(7) Chappell, M. D.; Stachel, S. J.; Lee, C. B.; Danishefsky, S. J. *Org. Lett.* **2000**, 2(11), 1633-1636.

(8) Prié, G.; Thibonnet, J.; Abarbri, M.; Duchêne, A.; Parrain, J. *Synlett* **1998**, 839.

(9) Reviews: (a) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* **1995**, *28*, 446; (b) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18; (c) *Alkene Metathesis in Organic Chemistry* Ed.: Fürstner, A.; Springer, Berlin, **1998**; (d) Fürstner, A. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 3012; (e) Schrock, R. R. *Top. Organomet. Chem.* **1998**, *1*, 1.

(10) (a) Keck, G. E.; Yates, J. B. *J. Am. Chem. Soc.* **1982**, *104*, 5829; (b) Review: Curran, D. P. *Synthesis* **1988**, Part 1, pp 417-439; Part 2, pp. 489.

(11) Exposure of epothilones **19** and **23** to nude mouse and human plasma led to degradation of **23** within 30 minutes, while epothilone **19** remained mostly intact (see Ref. 2 for details).

1